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DISCOVERY

The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Vol. 20

Fall 2019



*Dietetics Student of the
Year Making a
Difference Locally With
Research*



DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System



UNIVERSITY OF
ARKANSAS

Dale Bumpers College of
Agricultural, Food and Life Sciences

DISCOVERY

The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Vol. 20

Fall 2019

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Cover: Features the research of Bumpers student Laura Wasson, a Human Nutrition and Hospitality Innovation major, who looked at increasing low-income residents' access to fresh produce through a local mobile pantry. Laura was presented with the Arkansas AND Outstanding Dietetics Student Award at the state meeting of the Arkansas Academy of Nutrition and Dietetics in Little Rock, April 2019.

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Increasing low-income residents' access to fresh produce
through a local mobile pantry

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Letter from the Dean

As Dean of the Dale Bumpers College of Agricultural, Food and Life Sciences, I am continuously impressed by and pleased with the passion of our students and their motivation to answer questions, solve problems, bring attention to issues and truly make an impact in their various fields of interest.

Our purpose is to serve the people of our state, across the country and around the world. With our innovative programs, expert faculty and outstanding students, we will continue to do everything we can to improve the quality of life for everyone as our students become scientists, innovators, managers, policymakers, entrepreneurs, educators, and most importantly – difference makers.

Our students are preparing for successful careers, conducting impactful research, and sharing knowledge. The Discovery undergraduate research journal highlights the efforts of just a few of our talented students. Many of our outstanding faculty work with them to produce what you see here.

In this issue, you'll find results of projects by students and their mentors from the Department of Agricultural Economics and Agribusiness, the Department of Animal Science, the Department of Crop, Soil and Environmental Sciences, the Department of Food Science, and the Human Nutrition and Hospitality Innovation Program in our School of Human Environmental Sciences.

We encourage undergraduate research by awarding undergraduate research grants. Our students compete for research and travel grants awarded by the University of Arkansas Honors College and the Arkansas Department of Higher Education SURF grants program.

Projects may be designed to meet requirements for an honors project in the Bumpers College Honors Program. One of our goals is to prepare students to be responsible leaders with strong communication skills and problem-solving abilities. Inside this issue, you will find studies that highlight and exemplify those qualities in our student researchers and future leaders.

Congratulations to the student authors on completing your project. And thank you to the faculty mentors and editors who worked with them to make this collection possible. As a college, we are pleased and proud to present their work in a citable publication as a service to them and our readers.



Deacue Fields

A handwritten signature in black ink that reads "Deacue Fields".

Deacue Fields, Dean
Dale Bumpers College of Agricultural, Food
and Life Sciences

A Message from the Associate Dean of the Honors College and Co-chair of the Service Learning Initiative

Service learning is a teaching method that combines learning goals and community service to affect growth both among students and within society. In its five-year existence at the University of Arkansas, the Service-Learning Initiative has been charged with organizing and expanding service-learning-based course offerings. With support from the Honors College, we have expanded those efforts to include service-learning research opportunities. In my dual roles as associate dean of the Honors College and co-chair of the Service-Learning Initiative, I assist faculty and students in discovering connections between learning and community need. I also help students earn recognition for service learning on their transcripts and identify potential funding sources for their research.

Bumpers College faculty are leading students in strong academic research projects that address community needs, both locally and around the world. This issue of *Discovery* highlights three such projects. Laura and Bailey's research projects took on food insecurity—one project in Washington County, Arkansas, and the other 2,200 miles away in Dangriga, Belize. Belize also served as a backdrop for Kelsey's research, which focused on small mammal identification in an effort to develop a baseline inventory for researchers at a national park.

These three projects show that service-learning research possibilities are wide-ranging, depending on community need, student skill set and desired location. Further, interacting with community partners (whether human or small mammal) often results in unforgettable, life-changing experiences for the students. Any individual research project may not completely solve a great problem like food insecurity, but these experiences can move the needle closer to a permanent solution while having a positive impact on both the student and the community.

Thank you, university faculty and staff, Seeds that Feed, Billy Barquedier National Park and Derek Jones for guiding our students through these research projects (as the saying goes, it takes a village). Thank you, *Discovery* editors, for helping the students share these experiences with the world. Finally, thank you, students, for your dedication to these sometimes unconventional research experiences. You're showing Arkansans what we already know here at the University of Arkansas: that when the university and communities come together to address a need through research, great benefits can be generated for all.



Jennie Popp

A handwritten signature in black ink that reads "Jennie Popp". The signature is stylized with a large, looped "P" and a cursive "J".

Jennie Popp, Professor, Associate Dean of the Honors College, and Co-chair, Service Learning Initiative.

Discovery on ScholarWorks@UARK

Journal management and submissions are now facilitated through ScholarWorks@UARK, the institutional research repository for the University of Arkansas:

<https://scholarworks.uark.edu/discoverymag/>

Bumpers College undergraduate student research now reaches a worldwide audience via this powerful database, with its extensive search engine and analytics, and ease in downloading individual articles, we are already seeing the results. Here's a peek at readership distribution across the globe and most popular *Discovery* articles by download in recent months.



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Greenhouse aquaponics: Custom aquaponic systems at home

Meet the Student-Author



Jesse Blanchard

Research at a Glance

- Consumers nationwide are becoming more conscientious of where their food comes from and demand locally grown or environmentally friendly products.
- Aquaponic systems offer high-quality fruits and vegetables along with fresh fish proteins at home.
- "Do it yourself" (DIY) methods provide wide ranges of availability in production and aesthetic properties for all interested producers.

Growing up on an equestrian farm with a range of explorable land having both terrestrial and aquatic life, I have always been interested in natural systems—especially below water. This is due in part to learning to fish as a child backpacking with my father, Mike, who continued to encourage this hobby and even landed me an internship opportunity on a fish farm at Bell Aquaculture while still in high school. With a combination of technical skills learned at home and from the Prairie Grove agricultural program paired with the new knowledge of fish farming operations, the ideas for small scale aquaculture began. I pursued an agribusiness marketing and management major with a minor in Sustainability at the University of Arkansas. These areas of study along with the opportunity to conduct funded research through the Honors College allowed me to construct and operate my first aquaponic system as a means of gaining research and physical experience in what I believe may be a possible future in producing fruits and vegetables. I would like to thank my thesis mentor, Dr. David Hyatt along with my committee members Dr. Jennie Popp and Leah English for guiding me through the research portion of this invaluable project. I would also like to give special thanks to Mike Blanchard, John Blanchard, and Alex Fisher for their help in constructing and improving the system and its greenhouse shell.



Jesse holding golden shiners from his aquaponic project on his family's farm in Prairie Grove, Arkansas.

Undergraduate Research Articles

Greenhouse aquaponics: Custom aquaponics systems at home

Jesse Blanchard^{}, David G. Hyatt[†], Jennie Popp[§], and Leah English[‡]*

Abstract

Taking advantage of inherent natural systems, aquaponic practices hold the potential to serve as an educational, sustainable, and profitable hobby for home gardeners facing common constraints such as temperature, space, and pests. The goal of this research was to assess the feasibility of implementing a small scale (4542-L) home-based aquaponic system in a small (48.768 m²) greenhouse to produce fresh produce and fish protein. System construction and maintenance costs were compared to the value of crops and fish produced to determine whether this aquaponic system is a feasible option for the home grower. It was hypothesized that this system would break even in five years. Results showed that such a system can be successfully built and operated to yield fresh produce, fish protein, and a high value composted fertilizer on an annual basis. However, the payback period for the system can be five years or even longer, depending on the estimation of future costs and benefits and discount rates used. Results and experience from the greenhouse system have been and will continue to be used for system improvements, education on natural systems, designs for others, as well as a guide for aquaponic systems moving forward.

^{*} Jesse Blanchard is a May 2019 Honors program graduate with a major in the Department of Agricultural Economics and Agribusiness.

[†] David G. Hyatt, faculty mentor, research associate professor, Walton College of Business.

[§] Jennie Popp, professor, Department of Agricultural Economics and Agribusiness.

[‡] Leah English, technical assistant II, Department of Agricultural Economics and Agribusiness.

Introduction

Aquaponics can be defined as “the cultivation of fish and plants together in a constructed, re-circulating ecosystem utilizing natural bacterial cycles to convert fish wastes to plant nutrients” (Sawyer, 2010). This process is the combination of aquaculture and hydroponics which can be thought of as fish farming and soilless gardening, respectively. By first taking advantage of *Nitrosomonas* sp. bacteria in order to convert the ammonia from fish waste into nitrites (NO_2) aerobically and then into nitrates (NO_3) with *Nitrobacter* sp. bacteria, the ammonia which is toxic to fish is efficiently converted into a readily available solution for healthy plant growth (Backyard Aquaponics, 2012).

The goal of this research was to assess the feasibility of implementing a small-scale, home-based aquaponic system to produce fresh produce and fish protein. System construction and maintenance costs were compared to the value of crops and fish produced to determine whether this aquaponic system is a feasible option for the home grower. It was hypothesized that this 4542-L system would break even in five years.

Materials and Methods

System Design

Unlike James Rakocy and a team at the University of the Virgin Islands who constructed what is regarded as the first successful commercial system, much of the aquaponic world revolves around “do it yourself” (DIY) style builds (Rakocy, 1989). An online search yields abundant sources for custom designs that the average person with a few minor technical skills in construction and plumbing can manage. For this project, the greenhouse system was designed completely from DIY methods devised and implemented on the go, in order to add the crops desired by the family (Michell, 2019).

The greenhouse system included: the construction of both a small (48.768 m^2) greenhouse (Fig. 1) as well as a 4742-L aquaponics system: one 2271-L lined sump tank below ground, one 1135-L and one 378-L Rubbermaid water troughs as fish tanks, three 208-L drums cut in half with bell siphons and filled with pea gravel, 7.62 m of 7.62 cm PVC with pea gravel-filled net pots, and one $0.914 \times 2.438\text{-m}$ lined wooden box with Styrofoam rafts



Fig. 1. Greenhouse structure with horse manure compost heater and raised garden beds for transplanting larger plants.

complete with pea gravel-filled net pots. This system was completed in approximately 55 man-hours total between three people from March through June 2018.

The sump tank acted as the lowest point in a system where water is pumped up from and returned to by gravity. The sump tank received oxygen from an air pump via air-stone as well as mesh bags filled with expanded clay to serve as surface area for the nitrifying bacteria to convert ammonia into nitrates. Fifteen young bluegill perch and pond snails caught from the landholder's pond were put into the covered sump to aid in breaking up solid waste accumulation on the bottom as well as to consume any mosquito larva present.

From the sump tank, nutrient-rich water was pumped up to six half 208-L barrels filled with pea gravel before exiting through bell siphons. These media-filled grow-beds were used to grow crops that required deeper root systems to hold up their stalks while also serving as a water oxygenation method (Fig. 2). Water fills the tank until it reaches the top of the standpipe where it creates a siphon that rapidly drains water out of the media down

to the break in the bell. This ensured that the plants did not become oxygen-starved and flooded as each grow-bed filled with water and drained every 20 to 30 minutes.

After flowing through the standpipes, the water drained into the 1135-L primary fish tank stocked with 130 tilapia purchased from the Tilapia Depot (Tilapia Depot, 2019). This simple tank received a similar air-stone as the sump tank to provide oxygen to the fish along with a PVC and Styrofoam cover to prevent the fish from jumping. Here, the water flow split into two paths: one pumped up to 7.62 cm PVC pipes for a continuous flow growth method, and the other overflowed into the lined Styrofoam raft system. The continuous flow pipes resembled an overhead square with holes containing pea gravel-filled net pots on the top before draining down into the 378-L fish tank. Mesh wire was stapled around the PVC square to offer growth room for cucumber and watermelon vines planted in the net pots. Although the 378-L tank was never used to house fish in the initial system, it worked as an added buffer in water volume before draining back into the sump via overflow.



Fig. 2. Bell Siphon housed in media filled drums with tomatoes, bell peppers, mint, and basil present.

Overflowing through a 2.54 cm PVC pipe from the 1135-L tank, the other water route flowed into a 15.24 cm deep lined wooden box with a standpipe overflow into the sump. Floating styrofoam rafts with holes cut out to place pea gravel-filled net pots for planting filled this box (Fig. 3). This section received the most nutrients as it was directly after the main fish tank and thus acted as a plant nursery where seeds were planted to be later transplanted into the bell siphon grow-beds. After transplanting the other crops, mint was allowed to take over this section to create a massive filter for the accumulating nutrients in the system.

System Maintenance

Overall, daily maintenance in the greenhouse system was minimal and included feeding fish, trimming plants, measuring water quality, and adding weekly approximately 227 L of water when evaporation rates were highest in late August. In year one, there were a few small

leaks in the system that were repaired with silicone sealant. After initial cycling, the water parameters settled into the ranges of 7 to 9 pH and <1 ppm ammonia, nitrite, and phosphate. These fall within optimal ranges for tilapia (Michell, 2019). Finally, the addition of three purchased common pleco (*Hypostomus plecostomus*) which were utilized as algae and waste removers, ladybugs caught near the greenhouse for aphid control, and a local bee-hive for easy pollination drastically reduced additional maintenance needs of the greenhouse system.

Fish and Plant Production Systems

Initially, 130 tilapia were stocked in the system to grow. Once harvested, they were replaced with 3000 fingerling golden shiners (Anderson Minnows, n.d.). Additionally, nine fancy koi and 20 goldfish were added along with cattails, Salvinia, and other aquatic plants for aesthetics. The three common *Plecostomus* remained in the system for algae control. The fish waste was composted, and with



Fig. 3. Styrofoam raft nursery with tomatoes, basil, peppers, mint, lettuce, and watermelon seedlings present in gravel-filled plastic net pots.

the addition of worms could be transformed into valued vermicompost to be used in gardens.

Tomatoes, cucumbers, bell peppers, jalapeño peppers, watermelon, broccoli, rocket lettuce, squash, basil, and mint were grown from seed placed into the system in July 2018. Harvest began in late August with the leafy greens and early October for the fruiting plants. Harvesting continued until March 2019 when many of the plants were removed/relocated for remodeling. Even with cool temperatures, the greenhouse encasing the crops allowed production to continue throughout the winter and ripe tomatoes were even picked in March.

Cost-Benefit Analysis

A cost-benefit analysis was conducted using actual system expenditures and revenues for 2018 and projections of costs and revenues for six more years. Note that estimations on costs are likely slightly overvalued to account for unknown issues that could be caused by weather, unforeseen damages, and replacement of equipment. Following Adler et al. (2000), the net present value (NPV) of the project was examined using discount rates of 4%, 6%, 8%, and 10% to determine: 1) whether the project broke even within the expected time period, and 2) under what range of discount rates the results held.

Results and Discussions

Current and Future Cost and Revenue Projections

System expenses totaled nearly \$8400 in 2018. Infrastructure and electricity needs topped the costs (Table 1). In future years, nominal costs are expected to fall greatly as infrastructure and most electricity needs (tied to tilapia production) are removed. Based on lessons learned in the first year, improvements to system design and a shift to cold-water-tolerant baitfish and fancy koi will be made to benefit the system and to lower utility costs from heating.

Ninety-six of the original 130 tilapia survived with an average length of 17.78 cm and were moved to a personal pond to grow out to 25 to 30 cm. At a price of \$3.25 per pound, mature tilapia will have an estimated value of \$144 (Walmart, 2019). The baitfish and composted fertilizer will provide \$1400 in revenue annually in future years (The Home Depot, 2019).

Crops produced in 2018 included tomatoes, jalapeño peppers, bell peppers, yellow squash, mint, and basil. Based on retail prices of \$2.39, \$2.39, \$5.99, \$2.39, \$14.00, and \$7.99 per pound, respectively, total crop value was almost \$695.00 (Walmart, 2019). The most successful plants were tomatoes, bell peppers, basil, and mint. The most robust crop, mint, yielded an estimated 20.5 kg within the first year.

Table 1. Estimated costs and values of production for the first six years of operation for the greenhouse system including improvements and added sources of valuable products planned in the coming years.

	Greenhouse system 6 year projection, nominal costs and benefits							Total 6 Years
	2018 ^a	2019	2020	2021	2022	2023	2024	
Production costs in \$								
Infrastructure	5296	-	-	-	-	-	-	5296
Labor	825	90	50	-	-	-	-	965
Management	30	30	30	30	30	30	30	210
Fish and plants	252	400	-	-	400	-	-	1052
Feed	150	150	150	150	150	150	150	1050
Electric utility	1693	420	420	420	420	420	420	4216
Water utility	46.80	50	50	50	50	50	50	347
Repairs	25	50	50	50	50	50	50	325
Improvements	120	400	100	-	-	-	-	620
Total	8391	1540	800	650	1050	650	650	13,733
Production benefits in \$								
Crops	695	1100	1100	1100	1100	1100	1100	7295
Tilapia fillets	144	-	-	-	-	-	-	144
Baitfish	-	440	440	440	440	440	440	2640
Fertilizer	-	960	960	960	960	960	960	5760
Total	839	2500	2500	2500	2500	2500	2500	15,839

^a Year beginning 1 July 2018.

Cost-Benefit Analysis

The net present value of the project was calculated based on actual and expected costs and benefits listed in Table 1. Based on these values, net present value ranged from a high of \$1001.34 (at a discount rate of 4%) to a low of -\$1048.61 (at a discount rate of 10%) over the full period of the projected study. When the discount rate is 4%, the system breaks even during its fifth full year in operation, as expected. This break-even year result holds until the discount rate is increased to above 5.85%. This result is expected as higher discount rates require large benefits related to costs in the early years of a project while lower discounts place a higher value on benefits that accrue later in a project period.

Conclusions

This study showed that an aquaponics greenhouse system can be designed, installed, and maintained successfully in a home environment. Further, it can yield sufficient amounts of fresh produce and fish protein for a family to replace much of the purchasing needs for those items. The cost-benefit analysis showed that only under low discount rates (<5.85%) did the system break even after five full years of production. However, this analysis provides the “pessimistic results” as the cost-benefit analysis likely: 1) overestimated both current and future costs as the “learning curve” continues on operations, and 2) underestimated benefits as there was no “premium” estimated on the sale of local products, and benefit estimates did not consider the non-market benefits of the aesthetics provided by the systems aquatic environment.

While difficult to replicate exactly, the extreme versatility of system designs behind aquaponic systems allows any interested and dedicated person access to an exciting method for producing homegrown fruits and vegetables alongside decorative or harvestable fish. Despite not yielding as much as projected in the first year due to cold temperatures, the first-year experience suggested the beginnings of a solid aquaponics operation able to care for itself with only minor maintenance in the coming years. Results and experience from the operation will be used to further

optimize the greenhouse system as well as design and implement similar systems to meet specific goals for produced items or styles.

Acknowledgements

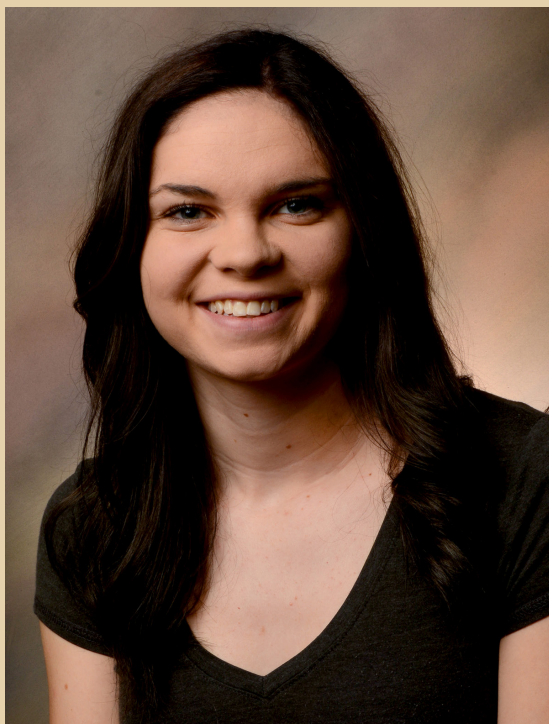
This research was made possible through funding by an Honors College Research Grant and a Bumpers College Undergraduate Research and Creative Project Grant as well as the construction location provided by Cedar Springs Equestrian Center in Farmington, Arkansas.

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Impact of selected infrared wavelengths on inactivation of microbes on rough rice

Meet the Student-Author



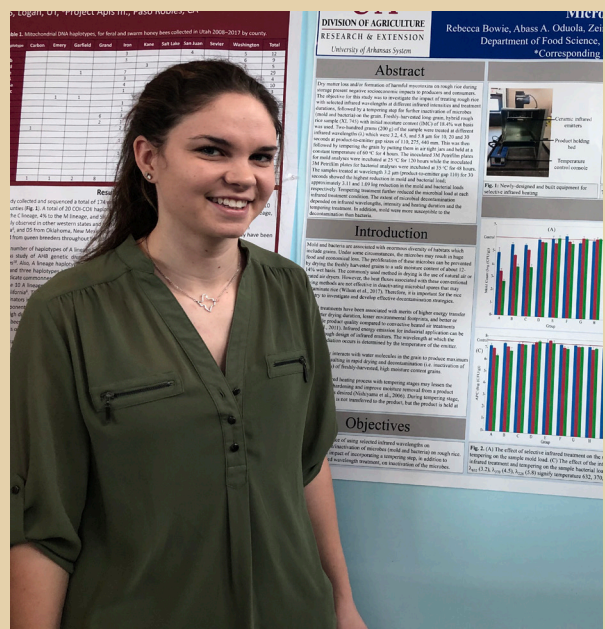
Rebecca Bowie

Research at a Glance

- Storage of rough rice in an inappropriate condition may lead to the growth of microbes such as mold and bacteria and may affect the quality and safety of the rough rice.
- Infrared heat treatment and tempering are effective in inactivating the microbes that may contaminate rough rice.
- The inactivation efficiency of infrared heat treatment and tempering is greater than that attainable by current heated air methods used in the industry and is more pronounced for mold than bacteria.

I am from Magnolia, Texas and graduated from Magnolia High School in 2016. I am currently in the Dale Bumpers College of Agricultural, Food and Life Sciences and plan to graduate with a degree in Food Science and a minor in Agriculture Business in May 2020. I interned with Simmons Foods as the Food Safety and Quality Assurance Corporate Intern in the summer of 2019. While interning, I researched the use of Whole Genome Sequencing for the analysis of microbial data within the poultry industry. Also in 2019, I studied abroad for a food sustainability program in Ghent, Belgium. There I was able to learn about successful water and food sustainability methods that they have implemented in the European Union. I have always had a strong desire to study food and enjoy learning more about food production and processes every day.

I am grateful for the help of my Honors mentor, Dr. Griffiths Atungulu, for allowing me to do research. He has helped me every step of the way and graciously shared his knowledge of rice with me. I would also like to thank my committee members, Luke Howard and Sammy Sadaka. Lastly, I would like to thank the following graduate students, Abass Oduola, Shantae Wilson and Zeinab Mohammadi-Shad for their collaboration and guidance throughout the research process.



Rebecca presenting her poster at the 2019 Honors Student Board Poster Contest. She was one of the very few undergraduate presenters.

Impact of selected infrared wavelengths on inactivation of microbes on rough rice

Rebecca Bowie^{*}, Abass A. Oduola[†], Zeinab Mohammadi Shad[§],
Shantae A. Wilson[‡], and Griffiths G. Atungulu[¶]

Abstract

The formation of harmful microbes and their associated mycotoxins on rough rice during storage presents negative socioeconomic impacts for producers and consumers. The objective of this study was to investigate the impact of treating rough rice with selected infrared (IR) wavelengths at different IR intensities and heating durations, followed by a tempering step for further inactivation of microbes (mold and bacteria) on the grain. Freshly harvested long-grain, hybrid, rough rice (RT CLXL745) with initial moisture content (IMC) of 18.4% wet basis (w.b.) was used. Two-hundred grams (200 g) samples of rice were treated at different IR wavelengths (λ), 3.2, 4.5, and 5.8 μm for 10, 20 and 30 seconds (s); at product-to-emitter gaps of 110, 275, and 440 mm. This was then followed by tempering the grain; putting samples in air-tight jars and holding at a constant temperature of 60 °C for 4 hours (h). Inoculated Petrifilm plates for mold and bacterial analyses were incubated at 25 °C for 120 h and 35 °C for 48 h respectively. Samples treated at wavelength 3.2 μm (product-to-emitter gap 110 mm) for 30 s showed the greatest reduction in mold and bacterial load; approximately 3.11 and 1.09 log reduction in the colony forming unit of mold and bacteria, respectively. Microbial analysis was performed on the rice prior to tempering, then all of the rice was tempered and microbial analysis was performed again to analyze the effectiveness of a tempering step. Tempering treatment further reduced the microbial load at each IR treatment condition. Molds showed more susceptibility to the IR decontamination than bacteria. This study provides useful information on the effectiveness of IR heating and tempering on microbial inactivation on rough rice.

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Introduction

Rice is known to be the primary food source for almost 50% of the total world population, thereby contributing about 20% of the total human dietary energy supply. In order to satisfy import/export demand and supply industries, huge amounts of rice are stored after harvest, often for more than a year (Fleurat-Lessard, 2017). When stored in an inappropriate condition, rice is susceptible to microbial contamination that directly or indirectly affects the quality and safety of the stored rice (Mohammadi Shad and Atungulu, 2019).

The proliferation of microorganisms on rice leads to musty odors, dry matter loss, discoloration, and accumulation of mycotoxin (Christensen and Kaufmann, 1969). This is as a result of the action of the spoilage microorganism interacting with themselves, with the grain, and with the environment of the storage facilities (Atungulu et al., 2018). The moisture content (MC) and temperature of rough rice are the two major parameters that influence microbial growth. Therefore, to prevent the proliferation of microbes, freshly harvested rice must be dried within a short duration to an MC of about 12–14% wet basis (w.b.).

The widely used conventional methods of drying employ the use of natural air or heated air dryers (Atungulu et al., 2019). Unfortunately, these conventional drying methods are ineffective in inactivating microbes and microbial spores that may contaminate the rice kernels (Wilson et al., 2017a). Therefore, it is of high importance to develop alternative methods of drying that can concomitantly dry and disinfect rough rice.

Infrared (IR) heating has been linked with the merits of higher energy transfer rate, shorter duration of drying, mild environmental footprints, and better or comparable product quality compared to convectively heated air treatments (Wang et al., 2011). In addition, IR heating has the potential to simultaneously dry and disinfect rough rice. For industrial applications, IR energy emission can be realized through design of IR emitters. The temperature of the emitter is used to determine the wavelength at which the maximum radiation occurs. Infrared can be classified into near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR) with wavelength ranges 0.75–1.4 μm , 1.4–8 μm , and 8–1000 μm , respectively (Krishnamurthy et al., 2008).

Generally, radiation penetration depth associated with IR heating is rather shallow. Therefore, IR treatment supplies high heat flux on the surface of the treated product. In the case of grain treatment, the IR heat dissipated on the surface of the grain may lead to case-hardening, surface discoloration or even burning before maximum moisture removal is achieved (Wilson et al., 2017b). Incorporating tempering steps may help to alleviate these challenges. The tempering process allows moisture redistribution through-

out the grain and eliminates moisture gradients generated during previous IR heating cycles; hence, it makes the next IR heating cycle effective in moisture removal (Li et al., 1998; Nishiyama et al., 2006). During the tempering stage, there is no transfer of IR energy to the grain, but the grain is allowed to rest at a constant temperature. Therefore, IR heating followed by a tempering step may have a higher potential to simultaneously dry and disinfect rough rice than just the application of IR heating.

This study aimed to investigate (i) the influence of using selected IR wavelengths on decontamination/inactivation of microbes (mold and bacteria) on rough rice and, (ii) the impact of incorporating a tempering step, in addition to selected IR wavelength treatment, on inactivation of the microbes.

Materials and Methods

The rice used was long-grain, hybrid, rough rice (RT CLXL745) obtained in 2016 from Poinsett Rice Inc., Waldenburg, Arkansas. Freshly harvested rough rice with initial MC of 18.4% w.b. was immediately cleaned using dockage equipment (MCi Kicker Dockage Tester, Mid-Continent Industries Inc., Newton, Kan.). The cleaned rice was put in tubs, sealed, and stored in a laboratory cold room set at 4 °C for 24 months. Twenty-four hours prior to conducting experiments, the rice was retrieved from the cold room and allowed to equilibrate with a room temperature of about 26 °C. The MC of the samples was determined by using an AM 5200 Grain Moisture Tester (PERTEN Instruments, Hägersten, Sweden) calibrated with a convective oven method.

A newly built, laboratory-scale IR system (Tempco Electric Heater Corporation) was used. The system consists of three ceramic emitters in one panel, heating chamber, product holding bed, and a temperature control console as shown in Fig. 1. The emitter has a metamorphic yellow (cold) to orange (hot) color. The equipment is made of low profile 20-gauge aluminized steel housing. The standard stocked voltage includes 220–240 V with watt density range from 17.1 kW/m^2 –54.3 kW/m^2 ; the temperature generated can be as high as 740 °C. This equipment produces IR radiation wavelengths of 3 to 6 μm .

The temperature console is used to vary the IR radiation wavelength generated. For instance, wavelengths of 5.8 μm , 4.5 μm , and 3.2 μm are produced at temperatures of 226 °C, 370 °C, and 632 °C, respectively. The wavelength was calculated using Wien's Displacement Law (Eq. 1).

$$\lambda_{\max} = \frac{b}{T}$$

Eq. 1

Where λ_{\max} is peak wavelength (μm), b is constant of proportionality ($2900 \mu\text{m}\cdot\text{K}$) and T is the absolute temperature in Kelvin.

A flat rectangular pan was covered with sterile aluminum foil and 200 g of rice samples were weighed into the pan and spread out to form a thin layer. Then, the thin-layered rice samples were put in the IR equipment and treated at selected wavelengths of 3.2 μm , 4.5 μm and 5.8 μm at three different product-to-emitter gaps (corresponding to different intensities) for different heating durations. The different intensities corresponding to each treatment combination is shown in Table 1. Three replications were done at each treatment combination level. After IR treatment, the samples were allowed to cool down to about 26 °C before they were carefully poured into sterile bags for microbial analysis. A control rice sample received no treatment.

Following the IR treatments described above, all samples were placed inside cleaned 16 oz. jars and covered tightly. The jars were then put in an incubator (Thelco Model 4, Precision Scientific Instruments, Inc., Chicago, Ill.) set at 60 °C for 4 h. After the incubation period, the jars were brought out and allowed to cool down to room temperature. The samples were carefully poured into sterile bags for microbial analysis. A control rice sample received no IR and tempering treatment.

Standard procedures for isolation, plating, and counting were employed (AOAC, 2002) to determine rice total microbial load. Phosphate-buffered dilution water (0.5 M, pH = 7.2) was prepared and autoclaved at 121 °C for sterilization (AOAC, 2002).

Microbial analysis was performed twice, once before tempering and then once after tempering. A 10-g sample of rice was weighed and placed into a sterile stomacher bag. Then, 90 mL of sterile phosphate-buffered dilution water was added to the stomacher bag and masticated. A lab masticator (Silver Panoramic, iUL, S.A., Barcelona, Spain) was used to dislodge the microorganism. The masticator was set at 240 s and 0.7 stroke/s. This process ensured that the rough rice samples were pulverized into powder for total microbial load analysis when mixed with dilution water. Serial dilutions were carried out by mixing 1 mL of the original mixture in the stomacher bag (first dilution– 10^{-1}) with 9 mL of sterilized phosphate-buffered dilution water in a test tube (second dilution– 10^{-2}) and so on until the sixth dilution (10^{-6}) was made.

The 3M Petrifilm Mold Count Plates and 3M Petrifilm Aerobic Count Plates (3M Microbiology Product, Minneapolis, Minn.) were used in enumerating mold and bacteria count, respectively. The plates were placed flatly in the bio-safety cabinet. The top film of the plate was carefully lifted

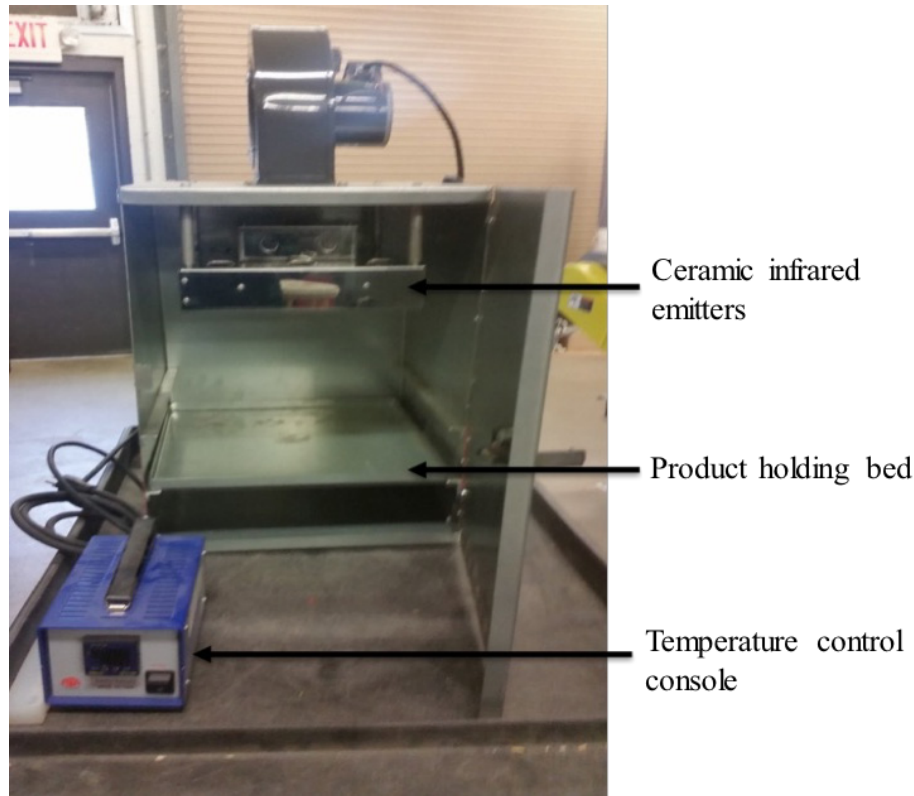


Fig. 1. Newly designed and built equipment for selective infrared heating.

and a P1000 micropipette (Finnpipette F2, Thermo Fisher Scientific, Inc., Vantaa, Finland), placed perpendicularly to the plates, was used to transfer 1 mL each of the sample solutions onto the center of the two 3M Petrifilm Plates (i.e., mold and aerobic plates). The top film was then gently lowered. The center of a plastic spreader was placed on the plates to align with the center of the plates. Light manual pressure was then applied on the plastic spreader to ensure even distribution of the inoculum on the Petrifilm plate. The gel was allowed to solidify for one minute. The inoculated Petrifilm plates with clear sides up were stacked to a maximum of 20 units and incubated.

The Petrifilm mold count plates and aerobic count plates were placed in an incubator (Thelco Model 4, Precision Scientific Instruments, Inc., Chicago, Ill.) at 25 °C for 120 h and 35 °C for 48 h, respectively, before counting. After the incubation periods, the colony forming units (CFU) on each plate were counted. Mold colonies on the plates appeared blue, black, yellow, or green, while bacteria colonies on the plates appeared red with a regular shape. The colony forming unit per gram (CFU/g) for each sample was obtained using Eq. 2:

$$T_{cfu} = \frac{P_{cfu}}{D_r} \quad \text{Eq. 2}$$

Where T_{cfu} is total colony forming units per gram of rice (CFU/g), P_{cfu} is colony forming units counted on plate per gram of rough rice (CFU/g) and D_r is dilution rate (10^{-1} to 10^{-6} times).

A statistical software JMP version 14.0.0 (SAS Institute, Inc., Cary, N.C.) was used to carry out analysis of variance (ANOVA) using fit model (full factorial analysis) and Tukey's honestly significant difference (HSD) test to determine significant differences within and among samples. All tests were considered to be significant when $P < 0.05$.

Results and Discussion

The initial mold load for the control samples was 5.74 log CFU/g. The effect of IR intensity and heating duration on the mold load of the samples is shown in Fig. 2. From the two-factor factorial analysis carried out, there was an IR intensity and heating duration interaction effect on the mean mold load of the samples. Only the highest three intensities (15.71, 10.08, and 7.27 kW/m²), all belonging to wavelength 3.2 μm, reduced the mold load of the rice samples. Greatest mold reduction was observed at the highest intensity (15.71 kW/m²) and longest heating duration (30 s) which brought about 3.11 log CFU/g reduction in the mold load. Other intensities belonging to wavelengths of 4.5 μm and 5.8 μm showed no reduction in the mean mold load of the samples regardless of the heating duration. Similar results with the current study were reported by Wilson et al. (2017b) where the IR heating of corn resulted in about 2.88 log reduction in the mold load. Also, Bingol et al. (2011) reported a 5-log reduction in the mold load of almond when treated with IR.

The effect of the IR treatment followed by the tempering step is shown in Fig. 3. The tempering step resulted in further reduction of the mold count after every IR treatment combination. All IR treatment combinations followed by the tempering step had significant effects on reducing the mean mold load of the samples. Compared to the IR treatment without tempering at the highest intensity of 15.71 kW/m² and longest heating duration 30 s, tempering further reduced the mold load by an additional 1.40 log CFU/g to bring the mold load reduction to 4.03 log CFU/g. In addition, for all the IR treatments that showed no significant effect, incorporating a tempering step led to a significant reduction in the mold load when compared to the control samples. For instance, the initial mold load of 5.74 log CFU/g was reduced to 5.53 log CFU/g after IR treatment at 0.73 kW/m² intensity for 30 s. However, incorporating a tempering step at the same IR intensity (0.73 kW/m²) and

Table 1. Experiment design of different combinations of infrared (IR) processing parameters.

Infrared heating duration (s)	Peak wavelength λ_{temp} °C (μm)	Product to emitter gap size (mm)	Intensity (kW/m ²)
10	λ_{226} (5.8)	110	1.55
		275	1.10
		440	0.73
20	λ_{370} (4.5)	110	4.13
		275	2.87
		440	1.86
30	λ_{632} (3.2)	110	15.71
		275	10.08
		440	7.27

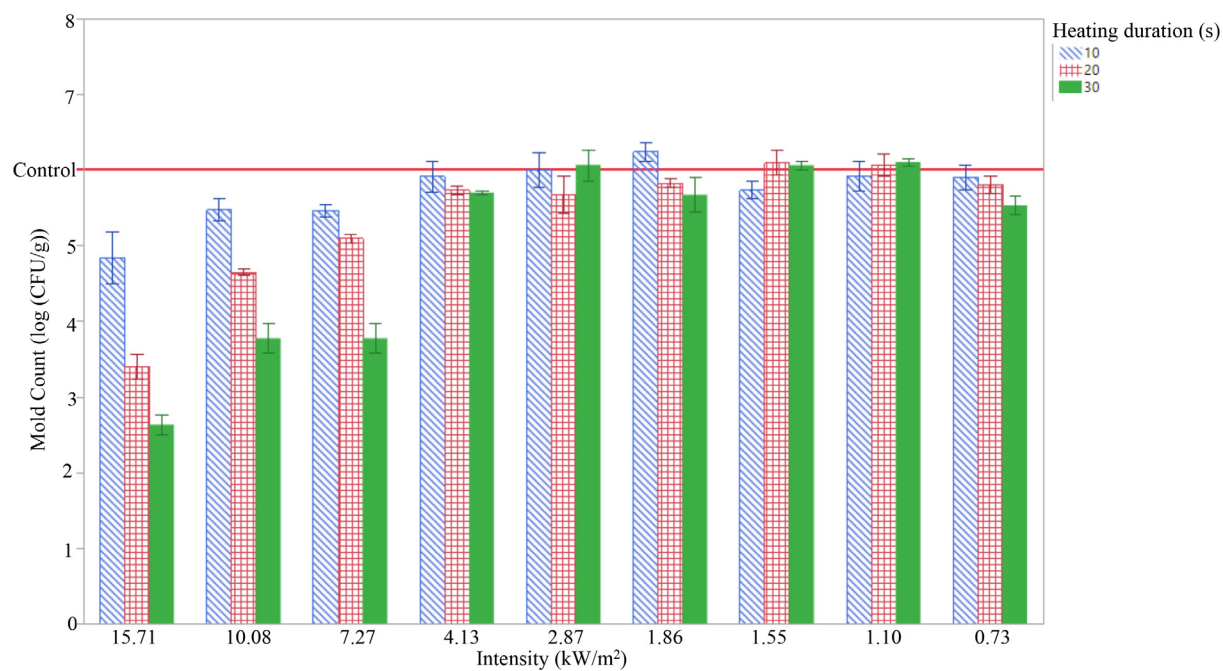


Fig. 2. The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s on the mold count on the rough rice samples; CFU signifies colony forming unit.

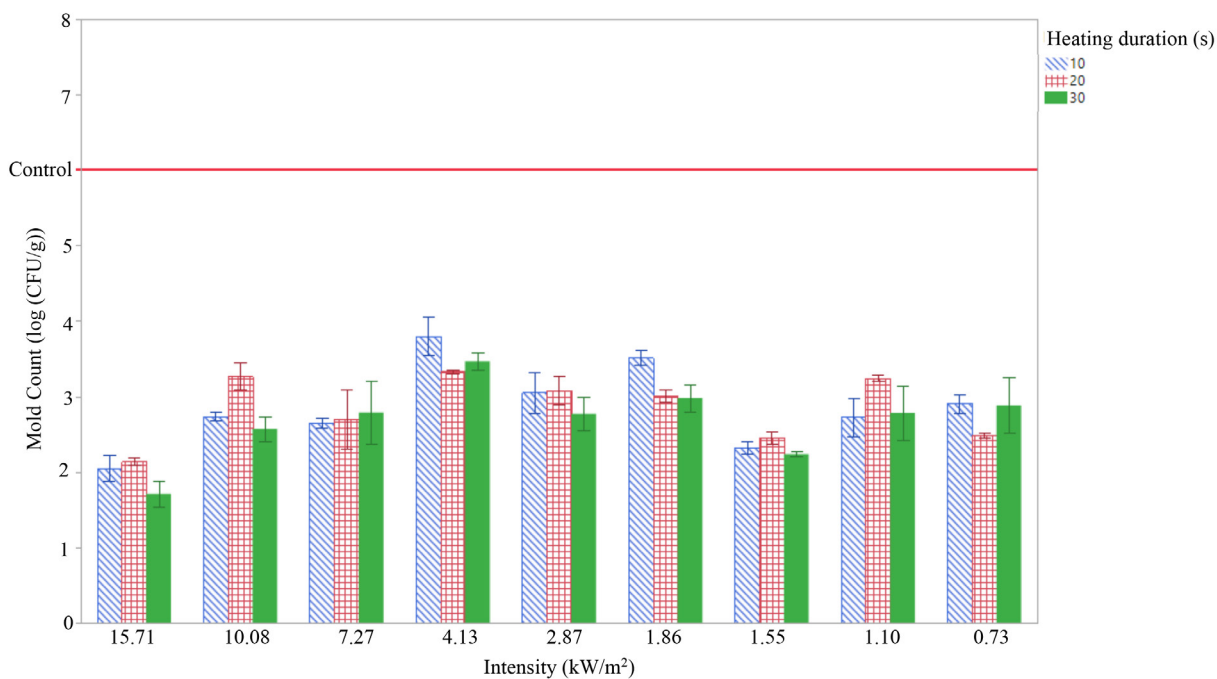


Fig. 3. The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s followed by tempering at 60 °C for 4 hours on the mold count on the rough rice samples; CFU signifies colony forming unit.

heating duration (30 s) statistically reduced the initial mold load to 2.88 log CFU/g. In agreement with the current result, Wilson et al. (2017a) reported that IR treatment of corn followed by tempering at 50 °C for 4 h resulted in 3.8–4.5 log mold reduction.

The effect of IR treatment on the aerobic plate count (APC) of the samples is shown in Fig. 4. The control samples had an initial APC of 7.44 log CFU/g. The IR treatment showed low efficiency in deactivating bacteria on the samples when compared to its effect on mold decontamination. Like the result of mold load, the highest intensity (15.71 kW/m²) at the longest heating duration (30 s) showed the maximum reduction; it brought the APC of the sample to 6.35 log CFU/g, i.e., a reduction of 1.09 log CFU/g. Other IR treatment combinations showed less reduction in the APC of the samples. Statistical analysis showed that the intensities of 15.71 kW/m² and 10.08 kW/m² had effects on the mean APC of the treated samples. The low reduction in APC by IR heating could be as a result of the presence of heat-resistant bacterial spores in the rough rice samples. A similar result was found by Staack et al. (2008), where they reported that IR heating resulted in a maximum reduction of 1 log CFU/g. In addition, Bingol et al. (2011) reported a very low reduction (0.62 ± 0.18) in the bacterial load when almond was treated using IR treatment. Mackey and Derric (1986) reported that the heat resistance of bacteria increased when bacteria were heated to elevated temperatures for a relatively short time.

Figure 5 shows the effect of the tempering step incorporated into the IR heating treatment on the APC of the

samples. Tempering caused a further reduction in the APC when compared to the samples treated without tempering. For instance, tempering the samples that were treated at an intensity of 15.71 kW/m² for 30 s brought about 3.50 log CFU/g reduction in the APC. Likewise, tempering the samples that were treated at an intensity of 0.73 kW/m² for 10 s brought about 1.52 log CFU/g reduction in the APC. Incorporating the tempering step made all the intensities that initially had no effects produce significant reductions in the mean bacterial load.

Conclusions

The IR treatment was effective in inactivating microbes (mold and bacteria) on rough rice. However, incorporating a tempering step led to further microbial inactivation. This treatment combination was more effective on mold inactivation than bacteria. Therefore, a longer heating duration may be required to further reduce the bacteria load on rough rice. For further study, these findings should be extended to inactivating aflatoxin-producing mold—*Aspergillus flavus*—to prevent the production and accumulation of aflatoxin on rice. In addition, the implication of the studied treatments on milled rice yields and quality characteristics should be evaluated.

Acknowledgements

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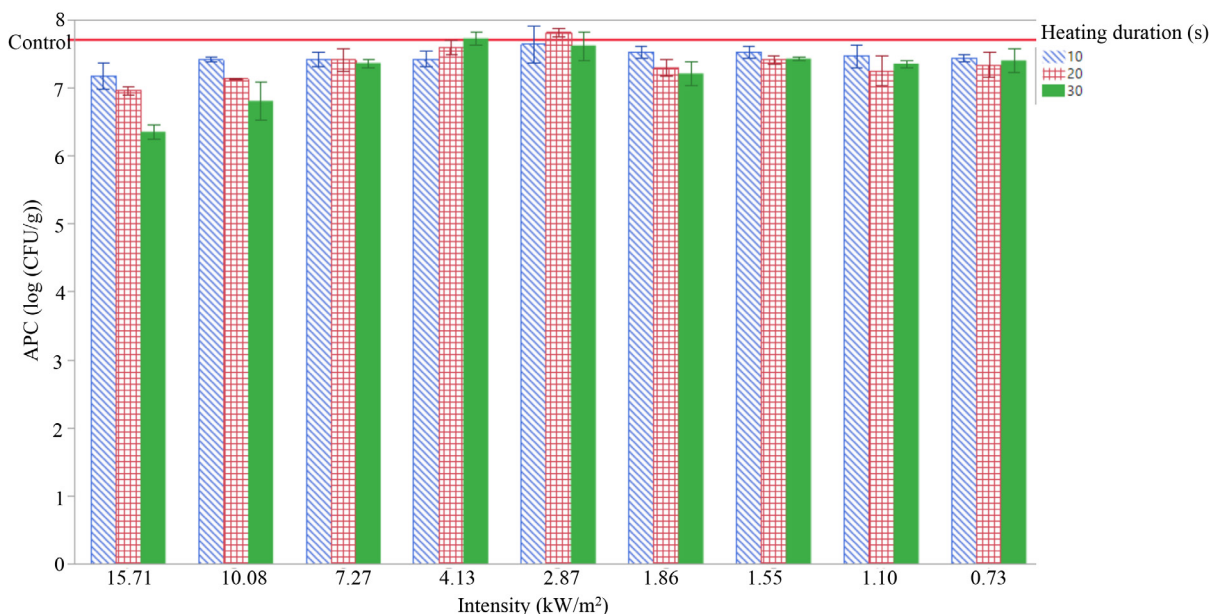


Fig. 4. The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s on the aerobic plate count (APC) on the rough rice samples; CFU signifies colony forming unit.

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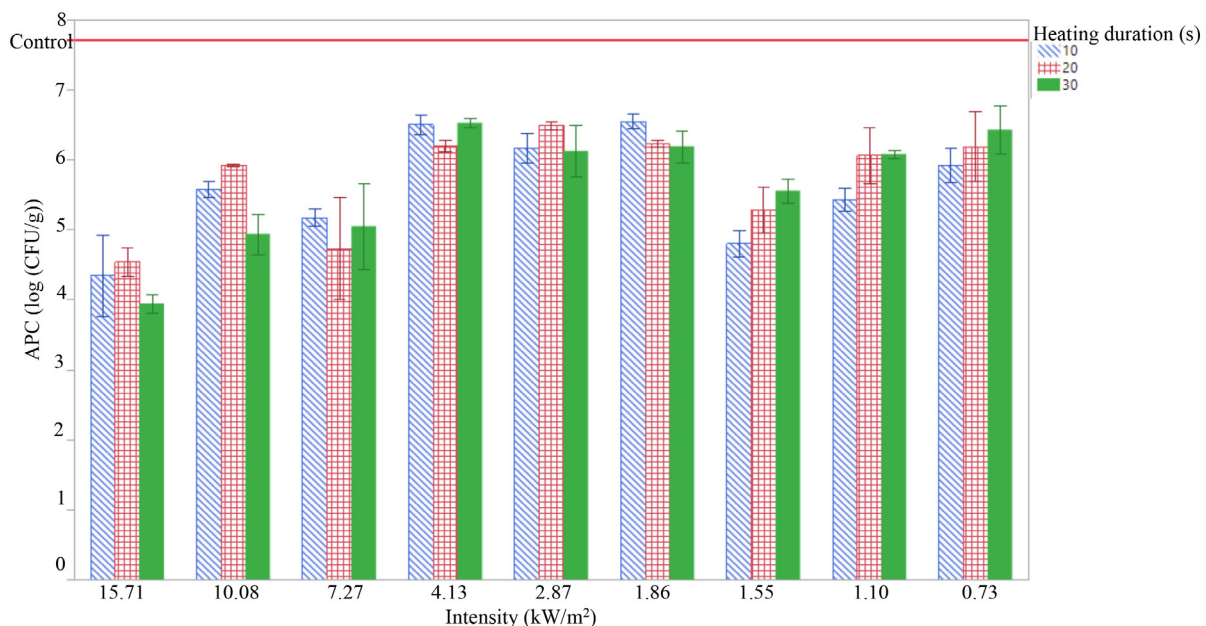


Fig. 5. The effect of infrared heat treatment at different intensities for different heating durations of 10, 20, and 30 s followed by tempering at 60 °C for 4 hours on the aerobic plate count (APC) on the rough rice samples; CFU signifies colony forming unit.

Observations and applications of husbandry methodologies on a backyard poultry farm in Dangriga, Belize

Meet the Student-Author



Bailey Carpenter

Research at a Glance

- There is a need to produce a manual for backyard poultry that can be easily accessible to those in developing regions attempting to improve their nutritional and economic standing.
- Biosecurity protocol, temperature regulation, and water and feed accessibility were the major factors observed regarding efficient poultry production.
- Improvement in biosecurity management and housing preparation could result in an overall increase in the productivity of poultry in a backyard setting with limited resources.

I grew up in Marine, Illinois showing horses and running cross country, while cultivating a love for the animal sciences. I graduated from Triad High School in the Spring of 2015 and began school at the University of Arkansas as a major in animal science with a pre-professional concentration. In the summer of 2016, I carried out poultry research in Nampula, Mozambique, and continued my research in the summer of 2018, where I spent two months in Dangriga, Belize collecting data for my honors thesis. I have also been an executive member for Hogs for Hope, a campus organization that benefits Arkansas Children's Hospital, by serving first as the marketing chair and later as overall director. I graduated Summa Cum Laude and was recently accepted into the University of Pennsylvania's School of Veterinary Medicine for the Fall of 2019, where I plan to receive my DVM degree and Ph.D. in avian pathology. I am beyond grateful for the guidance of Dr. Clark while I developed my passion for poultry medicine as a means to improve global development. I would also like to thank Dr. Rosenkrans and Dr. Farmer for the support they have shown me while pursuing research abroad and developing my honors thesis. Finally, I'd like to thank Dr. Ken Coffey, my academic advisor, for constantly pushing me to be the best version of myself.



Bailey in Nampula, Mozambique in the summer of 2016 working at the local poultry hatchery, New Horizons. Her research in Mozambique was where she developed an interest in improving poultry health as a means to improve global development.

Observations and applications of husbandry methodologies on a backyard poultry farm in Dangriga, Belize

Bailey Carpenter^{} and Fred Dustan Clark[†]*

Abstract

This study explores the husbandry methodologies on a backyard poultry farm in Dangriga, Belize, with the purpose of producing a set of guidelines for backyard poultry growers that have limited resources in similar regions. The majority of data collection occurred through survey questions approved by the Institutional Review Board (IRB)—which is responsible for protecting the rights and welfare of human research subjects—necropsies approved by an Institutional Animal Care and Use Committee (IACUC), and general observations. There has been a steady increase in poultry production in developing regions due to its positive effects on income and relative nutrition. However, due to a lack of accessible communication and education regarding effective and safe poultry production, these operators typically see poor productivity and/or profitability in their operations. Data were collected over biosecurity, vaccination protocol, water quality, feed quality, temperature regulation, housing set-up and preparation, and behavior for broilers and layers. Overall, the major factors that appeared to have the greatest impact on the birds were low biosecurity measures, low levels of clean available water, and consistently high temperatures experienced in the broiler pens. The results for each factor are discussed and it was suggested that if small adjustments were made, the birds would experience better health and therefore increased productivity. Additional studies regarding *E. coli* presence in water sources, trends in broiler weights, nutritional make-up of feed, and observations of trends in post-mortem findings should be conducted.

^{*} Bailey Carpenter is an honors program May 2019 graduate with a major in Animal Science with a Pre-Professional concentration.

[†] Fred Dustan Clark is the faculty mentor and a Professor in the Department of Poultry Science.

Introduction

The United States poultry industry has served as one of the leading influences in commercial operations due to its advanced technology and overwhelming level of resources. According to a report generated by Marin Weaver, the U.S. “is the largest poultry producer in the world, accounting for approximately one-quarter of global poultry production during 2006–12” (Weaver, 2014). Therefore, it is integral that production processes for broilers and laying hens be optimized to uphold the utmost level of efficiency. Because of this standard set for poultry management around the world, the United States is a major player in exports to countries such as China, Canada, Mexico, Indonesia, and Thailand (Weaver, 2014). In addition to this, the U.S. has also become the natural reference point to developing countries, such as Mozambique, that need to increase the level of nutrition in its inhabitant's diet and kick-start a stagnant economy through the production of chicken.

After spending just one month in Nampula, Mozambique, a general theme for developing countries became apparent: there is an increasing reliance on and significance of poultry. One Egg, a company run by Johnwayne Kennedy in Nampula, teaches people in the community that one egg a day can prevent severe malnutrition, an issue that is prevalent in one of three children in developing countries (Smith and Haddad, 2000). This concept of increasing protein in the diet is one that should be presented and stressed to all societies struggling to meet minimal standards for quality of life. Many individuals throughout these countries have begun to produce chickens in their backyards; however, it has come at a larger cost due to a lack of training and education (Fred D. Clark, pers. comm., 2018).

Derek Jones, a resident of Dangriga, Belize, is a trailblazer in the production of backyard poultry in his community. He has been working with the University of Arkansas for several years in an attempt to improve the economic, nutritional, and educational development of his community. However, this has come at a cost as he has not had the opportunity to evaluate an improve upon his own business. Mr. Jones produces and sells both broiler chickens and layer hen eggs, but he has recently seen a decline in his productivity. There is a rising prevalence of disease along with a subsequent rise in mortality, which appears to be a direct result of improper management protocols.

The problem Jones is facing is likely similar to those also attempting to produce chickens in their backyard. Instead of simply giving individuals from growing countries compensation to produce more birds, it would be much more practical to provide these communities with

useful instructions and proper education regarding poultry husbandry. Education would include topics such as biosecurity, vaccination/disease prevention protocols, egg temperature regulation, water quality, feed quality, and chick-housing preparation. Because the United States, and even more specifically Arkansas, is a leading producer of poultry, expertise is available to produce an instruction manual that could be accessible to those who have never before grown chickens. Not only would this manual seek to establish a stronger foundation for Belize, but if translated across several languages, it would also act to bridge the gap between the first and third-world countries.

Materials and Methods

The primary factors affecting poultry production were addressed by observing biosecurity protocol, water quality, feed quality, temperature regulation, and housing set-up and preparation. Each topic was evaluated through a series of survey questions approved by the Institutional Review Board (IRB), which were later administered to Derek Jones as a means to perform an in-depth analysis of his biosecurity and vaccination protocols, water and feed quality, temperature regulation, and housing set-up and preparation for the broilers and layers. Because Jones was the only individual responsible for his backyard operation, the survey was only given to him. Additionally, any observations that were pertinent to each subject matter were also recorded. To determine the relative effectiveness of the feed the chicks were receiving, 4 flocks of 8 birds each were weighed on their day of arrival, and on days 3, 5, and 10 of production. Each bird was weighed individually using a scale with the weight of the basket being subtracted from the total weight. For recording purposes, flocks were distinguished between each other through a color-coding scheme. Temperature regulation was analyzed using 4 Lascar EL-USB-2-LCD USB Data Loggers (Lascar Electronics, Inc., Erie, Penn.), which were placed in different locations throughout the yard at the beginning of the study and remained there until the end. The loggers collected temperature (in degrees Celsius) in 1-hour intervals throughout each day, and these data were then uploaded to a computer in the form of a graph to be analyzed later (Table 1). The temperature was monitored throughout June on 14 separate days for 24-hour intervals. To determine the possible prevalence of disease, or any other issues related to stunted growth and mortality, 8 necropsies were performed with a necropsy kit. Two broilers were necropsied on the 8th of June and both were from separate flocks. One of the birds was necropsied due to its inability to use its right wing, while the other bird was necropsied, because it was not gaining

weight like the others in its flock. The birds necropsied on the 12th and 20th of June were also incapable of gaining weight like the others in their flocks and were thus considered “throw-aways”. These birds were also from separate flocks. Two layers were necropsied on the 27th of June due to one layer being attacked by the owner’s dog and the other having an injured leg. Age of the layers could not officially be determined, but it was believed by Jones they were about two years old. Two broilers, both from the same flock, were necropsied on the 19th of July as a result of them being deemed throw-away birds. As instructed by F.D. Clark, each necropsy was performed with the same, methodical approach as a means to develop consistencies between the birds.

Results and Discussion

There was no separation of flocks from one another, other than a layer of chicken wire. From the time they entered and left the farm, each flock was in direct contact

with 2 other flocks within Jones’ operation. When rearing multiple flocks, they should be completely separated, from ceiling to floor, by the chicken wire, and should never share the same pen. Because each flock carries its own set of flora and potential-disease-causing pathogens, it is very important that they do not interact with other groups of birds (Swayne, 2019). Additionally, it could not be determined if the birds were being vaccinated prior to being sold to Jones. One flock, however, had injection marks for vaccines on their necks (Fig. 1) and were, on average, smaller than the birds that did not show signs of vaccination. It is believed that the birds showing no signs of vaccination were consistently larger because they were being shipped on day-of-hatch and were reaching farms immediately, allowing them to consume food and water. Alternatively, the birds being vaccinated were kept at the hatchery for a couple of days without adequate levels of food and water, thus decreasing their growth rate in the first couple of days and for the rest of the production period (F.D. Clark, pers. comm., 2018).



Fig. 1. Injection marks on the neck of a recently delivered chick.

Table 1. Average minimum and maximum temperatures recorded (in degrees celsius) for the four loggers placed throughout Derek Jones’ farm.				
	Logger 1- large nest box	Logger 2- Central Broiler Pen	Logger 3- Eastern-Most Broiler Pen	Logger 4- Western-most Broiler Pen
Average minimum temperature (°C)	28.0	28.4	30.2	27.9
Average maximum temperature (°C)	35.8	32.6	34.8	32.6

Throughout the study, it was observed that each Plasson Breeding Drinker contained a thick layer of mahogany shavings/litter (Fig. 2) where the birds would otherwise be able to access the water. The birds 0-1 week of age would reach up for the water, while those birds that were older than 3 weeks would have to bend their neck down to do so. Raising the waterers to a specific level (based on the flock's overall age) prevents the birds from kicking chicken litter into them. Chicken litter/feces can be covered in infectious pathogens and/or parasites and would, therefore, act as a very effective source for disease transmission. The constant maintenance and up-keep of water is crucial to the survival and successful production of broilers, as dehydration could otherwise result in such a hot and humid climate.

Typically, all of the feeders were checked (and possibly filled) around 9:00 AM each day and would not be checked/filled again until the subsequent morning. Because of this, 50% of the recorded instances showed the birds were without food. When the birds are constantly eating, they are also constantly growing. Thus, when Jones would leave some of his flocks without food (especially the chicks) for even a couple of hours, they were not growing in that period. Overall, this lowered the rate at which his birds were growing and the final weight they

would reach before harvesting (F.D. Clark, pers. comm., 2018). To ensure the birds always have feed available to them, the feeders should be checked every morning and every evening.

The lowest average minimum temperature recorded by a logger was 27.9 °C, which was located in the westernmost broiler pen. Thus, on average, the birds were experiencing a climate of at least 8.0 °C higher than recommended (Hulzebosch, 2005). Because of this heat stress, birds will consume less feed, as well as experience a reduction in egg weight and shell quality (Bell and Weaver, 2002).

Conclusions

The results of the study indicated that the major factors affecting efficient backyard poultry operations were biosecurity protocol, water quality, feed quality, and temperature regulation. If the backyard growers in Dangriga, Belize simulate the methodologies implemented in the United States and in other leading countries for poultry production, they should see an increase in productivity of their operation. By reaching an optimal range for poultry productivity, it is possible for these operations to provide a sufficient level of income and nutrition required to sustain a household.



Fig. 2. Plasson Breeding Drinker filled with bedding in broiler nursery pen.

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The effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on brown adipogenesis in stem cell culture

Meet the Student-Author

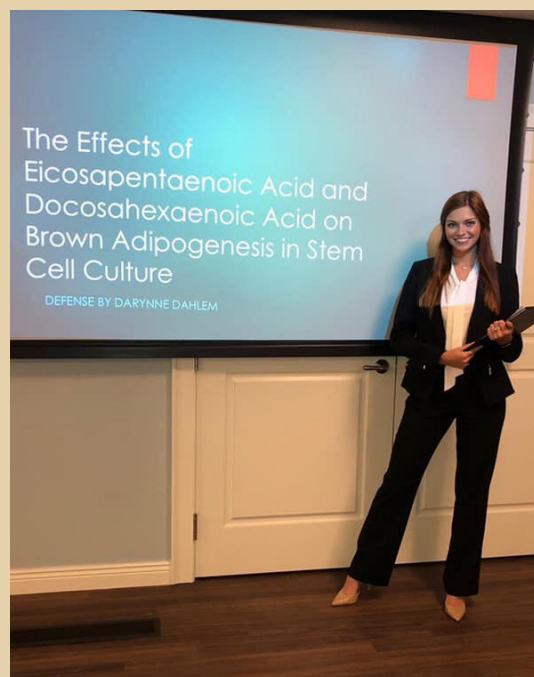


Darynne Dahlem

I am originally from Greenwood, Arkansas. I am a senior Animal Science major in the Bumpers College of Agricultural, Food, and Life Sciences. While studying at the University of Arkansas I have had the opportunity to be a part of Greek life as an active member of Delta Delta Delta, serve as an Associated Student Government Senator, and hold executive positions on the Bumpers College Honors Student Board. After graduation, I plan on getting a Master of Science degree at the University of Arkansas and then attend medical school to become a physician. In June, I was crowned Miss Arkansas 2019 and I will be competing in the Miss America competition on December 19th. I would like to thank Dr. Yan Huang for serving as my honors mentor and advisor for this project and I wish to recognize his constant support and fantastic teaching skills that helped me learn as much as possible throughout this experience. I would also like to thank Dr. Jason Apple, Dr. Charles Rosenkrans, and Dr. Jamie Baum for serving as committee members. I would also like to acknowledge Saeed Hemza for his help and technical assistance in completing this project.

Research at a Glance

- The objective of this study was to measure the effect of fish oil supplements on brown fat development.
- Stem cell culture was used as a model for this study.
- As a prenatal supplement, fish oil ingredients have a positive influence on fetal growth.
- The cell culture study indicated that fish oil inhibits muscle formation and promotes fat growth through genetic regulation.



Darynne at her thesis defense.

The effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on brown adipogenesis in stem cell culture

Darynne Dahlem^{} and Yan Huang[†]*

Abstract

Polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are major maternal dietary supplements due to their positive benefits on neurological tissue growth during the first 12 weeks of gestation. Previous studies show that EPA and DHA inhibit muscle formation but promote fat growth (adipogenesis). However, no research has addressed the question of whether a high intake of EPA and DHA affects brown fat development during gestation. The objective of this study was to measure the effect of EPA and DHA supplementation on brown adipogenesis and potential pathways related to mitochondrial biosynthesis using fibroblasts as an *in vitro* model. Using Oil-Red-O staining and polymerase chain reaction (PCR) testing, lipid droplet formation and six genes were examined. Results indicated that *PGC1 α* gene expression was affected by EPA and DHA treatment. Mitochondrial biosynthesis can potentially be promoted by increased *PGC1 α* gene expression with EPA and DHA supplementation. However, lipid droplet accumulation observed in the PUFAS-treated group indicated a previously unknown effect of n-3 PUFA supplementation on adipogenesis that needs to be further investigated.

^{*} Darynne Dahlem is a senior animal science majoring in Pre-professional Animal Science in the Department of Animal Science.

[†] Yan Huang, the faculty mentor, is an assistant professor in the Department of Animal Science.

Introduction

In the United States, rates of childhood and adolescent obesity have been on the rise for years and have nearly tripled since the 1970s. The Centers for Disease Control and Prevention (CDC) reports data taken from 2015–2016 show that nearly 1 in 5 school-age children and young people (6 to 19 years) in the United States are obese. There is increasing evidence that infants exposed to obesity-induced diabetes in utero have an increased incidence of childhood obesity and diabetes (Feig and Moses, 2011). Understanding the mechanism of the relationship between maternal and infant obesity is an urgent task in the study of childhood obesity.

Epidemiological and experimental studies show that food supplements, such as fatty acids, supplied to the fetus during pregnancy and to the newborn immediately after birth, can have long-term health effects on the development of metabolic diseases. These diseases include cardiovascular diseases, Type 2 diabetes, hypertension, and obesity. (Kabaran and Besler, 2015). Growing bodies of experimental studies indicate that reducing the risk of a variety of obesity-related diseases is strongly linked to an increase in the dietary supplementation and consumption of n-3 fatty acids (Seo et al., 2005). While a substantial number of studies have delineated many differences between the biological effects of saturated versus polyunsaturated fatty acids (PUFAs), less is known about the long-chain n-3 fatty acids commonly present in certain fish oils (Seo et al., 2005), such as eicosapentaenoic acid (EPA, 20:5,n-3) and docosahexaenoic acid (DHA, 22:6,n-3). Fish oil components, particularly two key biological regulators, EPA and DHA, appear to have the ability to modulate both cellular metabolic functions and gene expression. The synthesis of EPA and DHA from their 18:3 precursor α -linoleic acid is relatively inefficient, so meeting the body's need of n-3 fatty acids depends to a significant degree on the direct delivery of EPA and DHA with diet particularly from marine or industrial sources, such as fish oils. (Qi et al., 2002). Clinical research also showed that EPA and DHA supplemented during pregnancy accumulates in fetal tissues and cause a longer gestation.

Our previous studies showed that EPA and DHA inhibit muscle formation but promote fat growth, also called adipogenesis. However, no research has addressed the question of whether a high intake of EPA and DHA affects brown fat development during gestation. Brown adipose tissue (BAT) is an essential target in obesity prevention as well as treatment due to its ability to utilize fatty acids and glucose to generate heat by a mechanism not requiring muscle contraction, also called non-shivering thermogenesis. Most brown adipocytes originate from precursor cells in the

embryonic stage that express skeletal muscle marker genes and have similar mitochondrial features with muscle (Seo et al., 2005; Ong and Muhlhauser, 2011). In most eukaryotes, mitochondria are primary organelles that are responsible for energy metabolism derived from the breakdown of carbohydrates and fatty acids. It was reported that the n-3 PUFAs could cause higher oxidation levels of mitochondrial fatty acids in the myocardium (Flachs et al., 2005; Martins et al., 2012; Anderson, et al., 2014; Cavaliere et al., 2016). We hypothesize that EPA and DHA treatment impacts the brown adipogenesis of BAT precursor cells via metabolic changes in mitochondria. The objective of the current study is to measure the effect of maternal EPA and DHA supplementation on brown adipogenesis and potential pathways related to mitochondrial biosynthesis using fibroblasts as an in vitro model.

Materials and Methods

Cell Culture

National Institutes of Health (NIH) 3T3 fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a 5% CO₂ atmosphere. When cells confluency reached 90%, cells in the control group wells (Con, n = 6) were induced to brown adipocyte differentiation by switching to the differentiation medium 1 (DM1) containing 10% FBS, 1% penicillin-streptomycin, 170 nM insulin, 1 μ M dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 1 nM 3, 3',5-triiodo-L-thyronine sodium salt (T3), while 50 μ M EPA and 50 μ M DHA were added to DM1 in the fatty acid treatment group (FA, n = 6) for 3 days. Then Con cells were introduced to DM2 which only contained 10% FBS, 170 nM insulin, and 1nM T3. The DM2 in the FA group contained 50 μ M EPA and 50 μ M DHA. The DM2 with or without fatty acids was changed every 24 hours for 3 days.

Oil-Red-O Staining

Oil-Red-O staining was used to identify mature adipocytes. Cells were fixed in 10% formalin in phosphate-buffered saline (PBS) for 10 min at room temperature. Fixed cells were stained with the Oil-Red-O working solution for 7 min and rinsed with PBS to remove the excessive Oil-Red-O dye. The presence of Oil-Red-O dye in adipocytes was further quantified by measuring the optical absorbance at 520 nm.

Real-Time Polymerase Chain Reaction (PCR)

Gene expression related to brown adipogenesis, mitochondrial biosynthesis, and peroxisome biosynthesis was measured by quantitative real-time PCR. Total mRNA was extracted from cells with the TRIzol reagent (Fisher,

Pittsburgh, Pa.). The concentration of total RNA was assessed by Nanodrop OneC (Thermo Scientific, Waltham, Mass.), and quality was examined in the absorption ratio of OD260 nm/OD280 nm. The cDNA was synthesized from the RNA with iScript cDNA synthesis kit (Bio-Rad, Richmond, Calif.). Real-time PCR was carried out by using SYBR Green Supermix (Bio-Rad, Richmond, Calif.) on CFX Connect Real-Time PCR Detection System (Bio-Rad, Richmond, Calif.). The oligonucleotide primers used were designed with the NCBI database and Primer Quest (IDT.com). The primers (Table 1) were designed to target the genes: uncoupling protein 1 (UCP1), PR/SET domain 16 (PRDM16), iodothyronine deiodinase 2 (DIO2), peroxisome proliferator-activated receptor alpha (PPAR α), carnitine palmitoyltransferase 1beta (CPT1 β), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α). Each reaction yielded amplicons from 80 to 250 bp. The PCR conditions were as follows: 30 s at 95 °C, 30 s at 55 °C, and 40 s at 72 °C for 40 cycles. After amplification, a melting curve (0.01

°C/s) was used to confirm product purity, and the PCR products were electrophoresed to verify the targeted sizes. Results were expressed relative to β -actin. Data were analyzed using the $\Delta\Delta$ CT method, and the 18S gene was the reference gene.

Statistical Analyses

Differences between groups were assessed for significance by the unpaired *t*-test with the assumption of equal variances, and arithmetic means \pm SEM are reported. Statistical significance was considered as $P \leq 0.05$.

Results and Discussion

The Oil-Red-O staining showed lipid droplet accumulation in pre-adipocytes differentiated from 3T3 fibroblasts (Fig. 1A). The quantitative data showed that the red dye accumulated $20.04 \pm 6.95\%$ more ($P < 0.05$) in the FA group than in Con cells (Fig. 1B).

Table 1. List of primers.

Primers	Accession no.	Forward sequence	Reverse sequence
UCP1	NM_009463	CACGGGGACCTACAATGCTT	ACAGTAAATGGCAGGGGACG
PRDM16	NM_027504	AAGGAGGCCGACTTTGGATG	TTTGATGCAGCTCTCCTGGG
PPAR α	NM_011144	TGGTGTTCGCAGCTGTTTTG	AGATACGCCCAAATGCACCA
CPT1 β	NM_009948	TATAACAGGTGGTTTGACA	CAGAGGTGCCCAATGATG
PGC1 α	NM_008904	TCCTCTGACCCAGAGTCAC	CTTGGTTGGCTTTATGAGGAGG
18S	NR_003278	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGCG

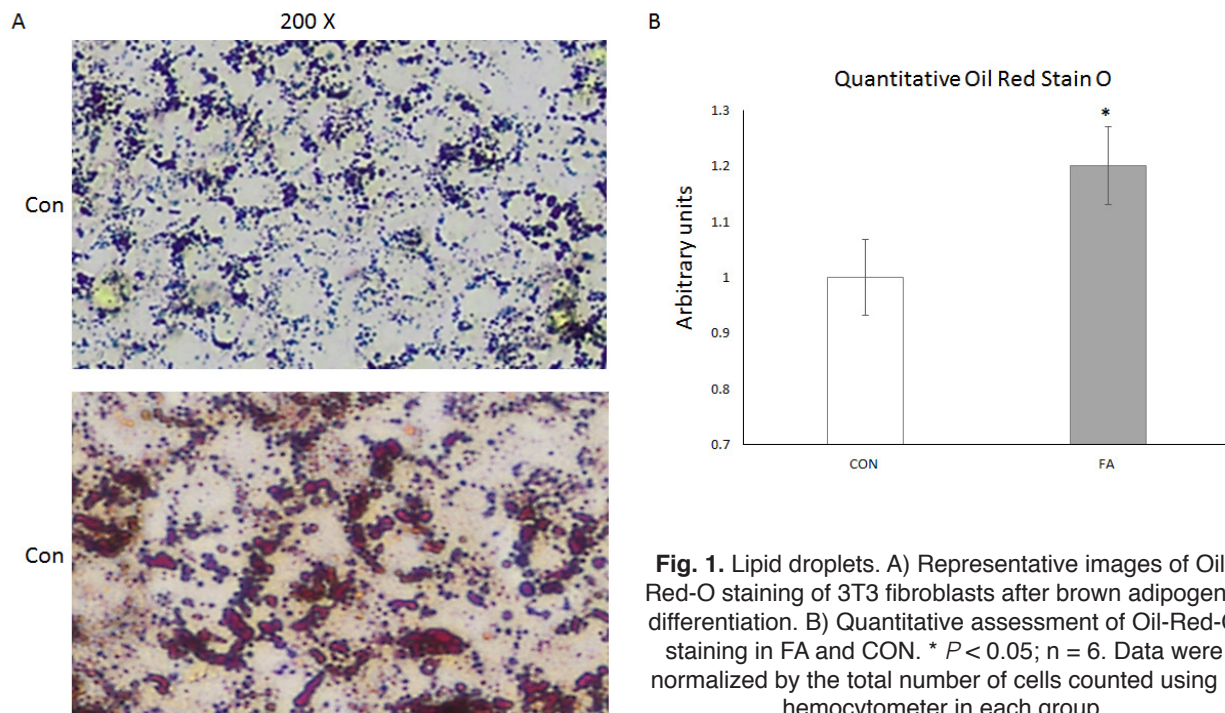


Fig. 1. Lipid droplets. A) Representative images of Oil-Red-O staining of 3T3 fibroblasts after brown adipogenic differentiation. B) Quantitative assessment of Oil-Red-O staining in FA and CON. * $P < 0.05$; $n = 6$. Data were normalized by the total number of cells counted using a hemocytometer in each group.

Among the brown and white adipogenic marker genes, the expression of PGC1 α was greater $31.81 \pm 5.17\%$ ($P < 0.05$; Fig 2) in the FA group than in Con cells. Other gene expression including UCP1, PRDM16, PPAR α , and CPT1 β was not different.

Long chain fatty acids are known to activate brown adipocytes (Gonzalez-Hurtado et al., 2018). In this study, the expressions of six genes were measured: UCP1, PRDM16, DIO2, PPAR α , CPT1 β , and PGC1 α . The UCP1 is known as uncoupling protein 1 and it works to separate oxidative phosphorylation from adenosine triphosphate (ATP) synthesis with energy dissipated as heat, it is also referred to as the mitochondrial proton leak and helps to reduce mitochondrial membrane potential in mammalian cells (Brondani et al., 2012). The PRDM16 is a protein-coding gene that has broad expressions in the stomach and thyroid among other tissues (Seale et al., 2008). Peroxisome proliferator-activated receptor alpha (PPAR α) increases the size and number of peroxisomes, which are subcellular organelles found in plant and animal cells that contain enzymes for respiration and for cholesterol and lipid metabolism (Choi et al., 2015). Carnitine palmitoyltransferase1 β (CPT1 β) is a protein-coding gene that encodes a protein that is the rate-controlling enzyme of the long-chain fatty acid beta-oxidation pathway in muscle mitochondria (Choi et al., 2015). The protein coded by PGC1 α is a transcriptional cofactor that regulates genes involved in energy metabolism (Austin and St-Pierre, 2012). Of these six genes, only one was affected by EPA and DHA supplementation. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α) is a transcription cofactor that functions as a master regula-

tor for many metabolic and physiological processes such as adaptive thermogenesis, glucose and fatty acid metabolism, muscle fiber type, and mitochondrial biogenesis (Flachs et al., 2005; Austin and St-Pierre, 2012). Overexpression of this transcription coactivator could improve mitochondrial function. It also could increase oxidative stress resistance. This upregulation could be an indicator that these long-chain fatty acids can increase the speed of the metabolic pathways when introduced to fibroblasts. However, it has also been recognized in recent studies that 3T3 cells are insensitive to both fatty acid and beta-adrenergic agonist stimulation. The 3T3 cells are the most commonly used because they have a high affinity for harboring lipids into the cytoplasm when stimulated. The understanding that they are insensitive to the treatment of long-chain fatty acids helps to explain the lack of statistical differences between the control group and the treatment group (Shin and Ajuwon, 2018). The results collected are a helpful piece in the equation that is prenatal nutrition. A limitation of this study is that only PCR and staining results could be presented. The results could be fortified by further testing the cell line for oxygen consumption rates, conducting Western Blot tests, and additional PCR analysis of genes involved with thermogenesis, mitochondrial biosynthesis, and protein synthesis. Another limitation was the sample size that survived until final testing.

Conclusions

Results indicate that mitochondrial synthesis has the ability to be induced through the introduction of certain

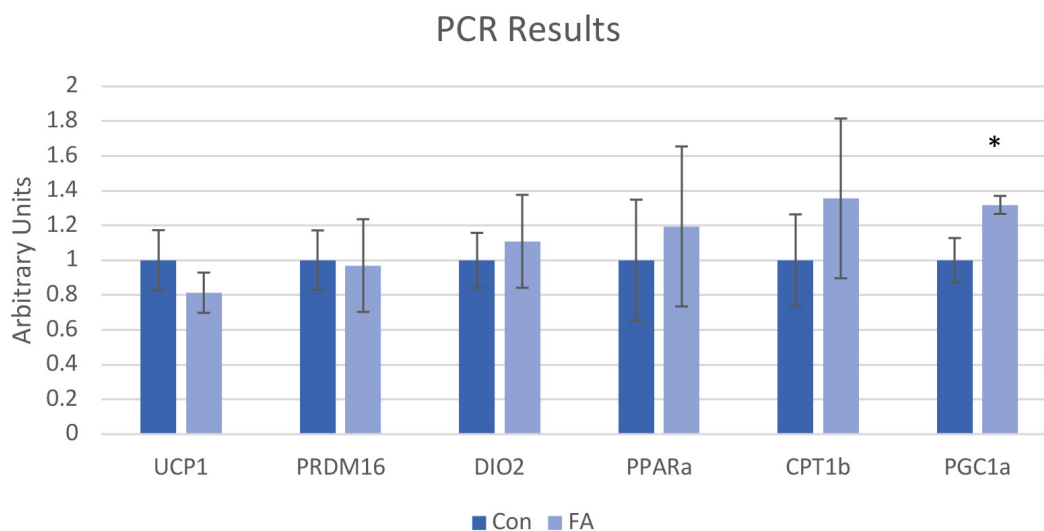


Fig. 2. Gene expression analysis by RT-qPCR of key genes related to adipogenesis, mitochondrial biosynthesis, and metabolism. Data are expressed as mean + SEM. The relative expressions were calculated in arbitrary units. * $P < 0.05$; $n = 6$.

long-chain fatty acids. This would usually be paired with smaller lipid droplets since higher mitochondrial counts allow for more rapid lipid degradation. However, the results of this study show greater lipid concentrations in the cells while also having greater mitochondrial counts. For this reason, additional studies are needed to understand the reason behind this discrepancy and to eventually realize the effect of EPA and DHA on adipogenesis in relation to thermogenesis and increase of obesity post-partum when introduced to fibroblasts in-vitro. The researchers suggest the use of a sturdier cell line that is easily stimulated by fatty acid treatment and to run more diagnostic testing focusing on mitochondrial biosynthesis.

Acknowledgements

This research was made possible by the Bumpers College Honors Faculty Committee's generous funding of this project.

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Anthocyanin stability in food products made with freeze-dried blueberry powder

Meet the Student-Author

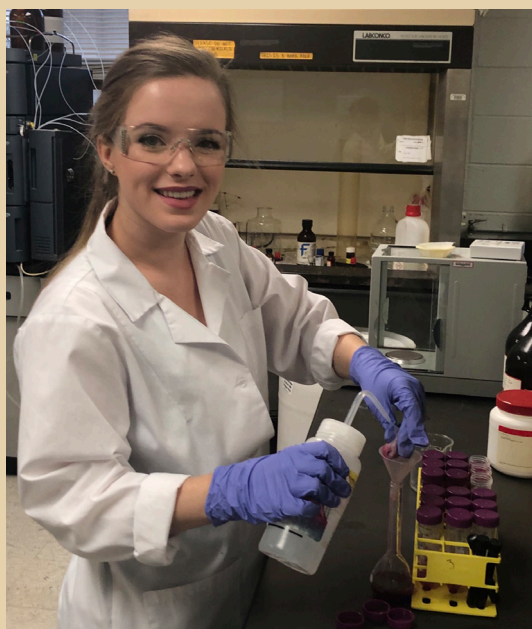


Samantha Findley

Research at a Glance

- This study evaluated the stability of six blueberry products (gummy, graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) prepared with freeze-dried wild blueberry powder.
- Food products were analyzed before and after processing and after two, four, six, and eight weeks of storage.
- The ice pop was the best product for shelf stability of chemical compounds found in blueberries in the food products evaluated.

I am from The Woodlands, Texas. While at the University of Arkansas, I was a member of the Student Dietetic Association and served as Secretary my senior year. I also served as a Bumpers College Ambassador for two years. I graduated Magna Cum Laude with Honors Distinction with a degree in food science and a degree in human nutrition and hospitality innovation. Through studying nutrition and food science I have gained an interdisciplinary understanding of food, both in its complexity alone, and how it affects the human body. While conducting this research, I have employed techniques I learned about in courses but never thought I would get the chance to utilize and have truly had a hands-on experience. I am fortunate to have had guidance throughout the entire process. I thank Cindi Brownmiller for spending countless hours teaching me lab techniques, helping me analyze my thesis data and answering a myriad of questions. I also thank my mentor, Dr. Luke Howard, for his confidence in me, continuous support, and for always having a teaching heart; and to Drs. Jamie Baum and Han-Seok Seo for serving on my committee. I am thankful for the honors college research grant that supported my research as it showed a willingness to invest in me. After graduation, I will begin a combined master's degree and dietetic internship program in St. Louis and continue on my path to a career as a dietitian.



Performing anthocyanin extractions on food products in Dr. Howard's lab in the Department of Food Science.

Anthocyanin stability in food products made with freeze-dried blueberry powder

Samantha Findley^{} and Luke Howard[†]*

Abstract

This study evaluated the stability of anthocyanins in six blueberry products (gummy, graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) prepared with freeze-dried wild blueberry powder during processing and over eight weeks storage. Total anthocyanins were determined by high-performance liquid chromatography (HPLC) before processing and at day 0 and 2, 4, 6, and 8 weeks of storage. Thermal processing of gummy and graham bar products resulted in losses of anthocyanins (50% and 31%, respectively). An eight-week storage time also resulted in a decrease in anthocyanins (7% to 51%) in products stored at ambient temperature. The ice pop, which was stored at -20 °C, was the best product for shelf-stability as it experienced no decline in total anthocyanins during processing or over the entire shelf-life study. Future research should be conducted to determine the differences in total anthocyanins in the products over time when they are stored under refrigeration. Additionally, polymeric color should be analyzed as this indicator has the potential to further explain the nature of the decrease in anthocyanins observed during storage.

^{*} Samantha Findley is an honors program May 2019 graduate with a major in food science, and human nutrition and hospitality innovation.

[†] Luke Howard, the faculty mentor, is a Professor in the Department of Food Science.

Introduction

Diet during childhood can influence bone mineral density and susceptibility to chronic disease later in life (Chen et al., 2010; Gilsanz and Wren, 2007). Population-based studies have shown fruit and vegetable intake is an independent predictor of bone size in children and may contribute to the building of bone mass (Novotny et al., 2004; Tylavsky et al., 2004; Lanham, 2006). Unfortunately, many children fail to consume the recommended amount of fruits and vegetables (Kuntz et al., 2015). The recent popularity of consuming fruits and vegetables in prepared forms is worth exploring as a means to increase the consumption of health-promoting compounds, like anthocyanins in blueberries (Kuntz et al., 2015). Additionally, anthocyanin-rich products are needed for human clinical trials that require subjects to consume large doses of anthocyanins each day.

Anthocyanins, a class of polyphenols, are responsible for the brilliant red, blue, purple and black colors of fruits and vegetables. The six major anthocyanins found in fruits and vegetables, delphinidin, cyanidin, petunidin, peonidin, pelargonidin, and malvidin vary according to the substituents (OH or OCH₃) attached at positions R₁ and R₃ on the B ring (Fig. 1). Anthocyanins in nature almost always have a sugar (glycoside) or multiple sugars attached at carbon three on the middle heterocyclic ring.

The anthocyanin content of berries varies distinctly from one type of berry to another (Lee et al., 2015). It

is thought that since the composition of anthocyanins in berries affects their bioavailability and antioxidant effects, different anthocyanin-containing berries may affect the same cells differently (Lee et al., 2015). Blueberries from various sources have been found to contain 20–27 different anthocyanins (Wu and Prior, 2005). Wild blueberries are an especially good source of the anthocyanins petunidin and malvidin, delivering 87.6 mg/100 g and 154.6 mg/100 g, respectively (Wu et al., 2006).

The average daily intake of raw blueberries has been estimated to be 0.93 g, yielding 3.39 mg of anthocyanins (Wu et al., 2006). Intake of greater than 100 mg of anthocyanins per day could easily be achieved with the regular consumption of blueberries (Wu et al., 2006). In an *in vitro* study, malvidin-3-glucoside, one of the major monoglucosides in wild blueberries, was found to be significantly more effective at inhibiting pro-inflammatory genes than epicatechin or chlorogenic acid (Esposito et al., 2014).

Degradation and loss of anthocyanins during processing is of great concern when developing processed blueberry products. Conditions during thermal processing must be considered to ensure the biological activity of anthocyanins is retained in a thermally processed product (Rodríguez-Mateos et al., 2014). Thermal processing has been found to result in total monomeric anthocyanin losses of 28% to 59% in canned, pureed, and juiced blueberry products (Brownmiller et al., 2008).

The objective of this study was to evaluate the stability of anthocyanins in six blueberry products (gummy,

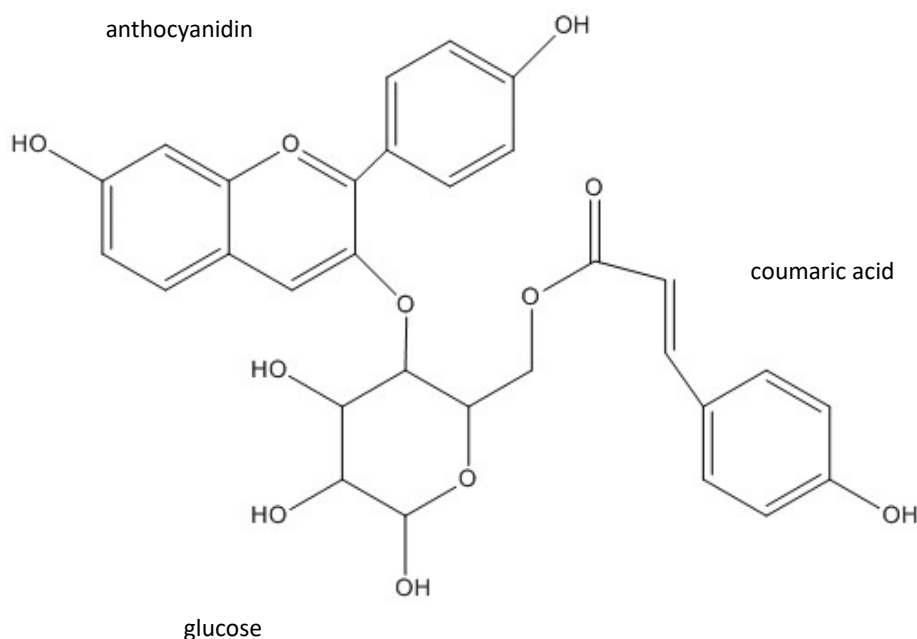


Fig. 1. The skeletal structure of an acylated anthocyanin.

graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) prepared with freeze-dried wild blueberry powder during processing and over eight weeks storage.

Materials and Methods

The freeze-dried powder used in the products and as a comparison was HiActives® North American wild blueberry powder 1.5% (FutureCeuticals, Momence, Illinois). Prepared blueberry products were previously formulated, produced, and packaged by others in the lab before the start of the shelf-life study. A 5-g sample for anthocyanin analysis was taken from each of three packages of each product (gummy, graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) at each timepoint during the shelf-life study. Timepoints were: day 0 (immediately after processing) and after 2, 4, 6, and 8 weeks of storage at 21 °C (except for the ice pop which was frozen and stored at -20 °C).

Anthocyanin Extraction and Sample Preparation

Samples of each food product, gummy, graham bar, oatmeal bar, and rice krispy bar, excluding the ice pop and juice, were first homogenized with methanol/water/formic (60:37:3, v/v/v) acid solvent using a Euro Turrax T18 Tissue-mixer (Tekmar-Dohrman Corp., Mason, Ohio; Brownmiller et al., 2008). Homogenized samples were filtered through Miracloth (Calbiochem, La Jolla, Calif.) and the filtrates were adjusted to a volume of 150 mL in a volumetric flask (Brownmiller et al., 2008). The 150-mL filtrate solutions were poured into beakers to ensure adequate mixing of the filtrates and transferred from the beakers into labeled 50-mL test tubes. From the 50-mL test tubes, 5 mL was pipetted into separate labeled 50-mL test tubes. The test tubes containing 5 mL of filtrate solution were dried overnight in a Speed Vac® concentrator (ThermoSavant, Holbrook, New York) and reconstituted with 1 mL of aqueous formic acid solution (Cho et al., 2004).

Ice pop and juice samples did not require the same extraction method. Both pasteurized and unpasteurized juice samples were evaluated to measure the anthocyanin losses during thermal processing of the juice. The ice pop and juice samples were diluted (200 µL ice pop or juice + 800 µL 5% formic acid) in glass test tubes prior to high-performance liquid chromatography (HPLC) analysis. The reconstituted extracted solutions from the products and the diluted juice and ice pop samples were each passed through 25-mm syringe filters with 0.45 µm nylon membranes (VWR International™, Radnor, Pennsylvania) before analysis by HPLC.

High-Performance Liquid Chromatography

Chromatographic analysis was performed using an established HPLC method to measure the content of in-

dividual anthocyanin monoglycosides (Brownmiller et al., 2008). Anthocyanin analysis by HPLC was performed according to the method of Cho and coworkers with a 50-µL injection volume of samples using a Waters HPLC system (Waters Corp, Milford, Massachusetts) fitted with a model 600 pump, 717 Plus autosampler, and a model 996 photodiode array detector (Cho et al., 2004). For separation, a 4.6 mm × 250 mm Symmetry® C18 column (Waters Corp, Milford, Massachusetts) and a 3.9 mm × 20 mm Symmetry® C18 column (Waters Corp, Milford, Massachusetts) were utilized (Cho et al., 2004). A linear gradient of 5% formic acid and methanol was used for the mobile phase (Cho et al., 2004). Detection wavelength used for the anthocyanins was 510 nm (Cho et al., 2004). Identification of the anthocyanins in the freeze-dried blueberry powder was previously performed by HPLC-MS. Individual anthocyanin glycosides were quantified as delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside equivalents using external calibration curves of a mixture of the five anthocyanin glucosides. The concentrations of individual anthocyanins were summed and reported as total anthocyanins. Results are expressed as total milligrams anthocyanins per gram of blueberry powder in each product.

Statistical Analysis

Statistical analysis was performed using JMP® software (SAS Institute, Inc., Cary, North Carolina). One-way analysis of variance with mean comparison by student's *t*-test was used to determine significant differences ($P < 0.05$) in average total milligrams anthocyanins per gram of blueberry powder in each product during the eight-week shelf-life study to measure anthocyanin stability over time. Significant differences ($P < 0.05$) in average total milligrams anthocyanins per gram of blueberry powder in each product at day 0 were also compared to average total milligrams anthocyanins per gram of blueberry powder to evaluate the stability of anthocyanins during processing of the products using a student's *t*-test.

Results and Discussion

Effects of Processing

Compared to the unprocessed blueberry powder, there was no difference ($P > 0.05$) in the average milligrams anthocyanins per gram of blueberry powder in any of the prepared products at day 0 (after processing), except for the graham bar and the gummy (Table 1). There was also no decrease for the juice ($P > 0.05$) in total anthocyanins during pasteurization (Table 1). The graham bar contained 31% fewer anthocyanins after processing and the gummy contained 50% fewer anthocyanins after processing (Table 1).

Shelf-life Study

For the gummy, there was a relatively linear decrease in total anthocyanins during the shelf-life study. Between day 0 and week 8, a 51% decrease in anthocyanins was measured (Fig. 2). The total anthocyanins in the graham bar remained relatively stable from day 0 to week 8 (Fig. 3). There was no decline in anthocyanins until week 6 and a 7% decrease in anthocyanins was measured between day 0 and week 8 (Fig. 3). Total anthocyanins in the rice krispy bar at day 0 and week 6 and 8 were different ($P < 0.05$) (Fig. 4). From day 0 to week 8 there was a 34% decrease in total anthocyanins (Fig. 4). Total anthocyanins in the oatmeal bar decreased gradually over the course of the shelf-life study (Fig. 5). From day 0 to week 8, a 28% decrease in total anthocyanins was observed (Fig. 5). No decrease ($P > 0.05$) in anthocyanins in the juice

was measured until week 6 of the shelf-life study (Fig. 6). Between day 0 and week 8, the pasteurized juice experienced a 47% decrease in total anthocyanins (Fig. 6). The anthocyanins in the ice pop were stable with no change ($P > 0.05$) observed between day 0 and week 8 (Fig. 7).

The anthocyanin contents of the prepared food products were compared to that of the freeze-dried blueberry powder used to prepare the products. Because there was no difference ($P > 0.05$) in the average milligrams anthocyanins per gram of blueberry powder in any of the products except for the graham bar and the gummy when compared to the unprocessed blueberry powder, it appeared the methods used to produce the oatmeal bar, rice krispy bar, ice pop, and juice (both pasteurized and unpasteurized) do not cause significant losses of anthocyanins (Table 1).

Table 1. Total anthocyanin content of prepared blueberry products.

Product	Average mg anthocyanins per g blueberry powder in product	Decrease in total anthocyanins measured (%)
Powder ^a	19.55 ± 1.6 ^b	Reference
Gummy	9.7 ± 0.7	50%
Graham bar	13.5 ± 0.7	31%
Rice krispy bar	16.9 ± 1.7	NS ^c
Oatmeal bar	19.2 ± 0.6	NS
Juice, NP ^d	21.81 ± 0.4	NS
Juice, P ^e	21.44 ± 1.3	NS
Ice pop	22.6 ± 0.3	NS

^a Blueberry powder used to prepare blueberry products.

^b Values represent means ($n = 3$) ± SEMs.

^c NS = no significant difference ($P > 0.05$).

^d NP = not pasteurized.

^e P = pasteurized.

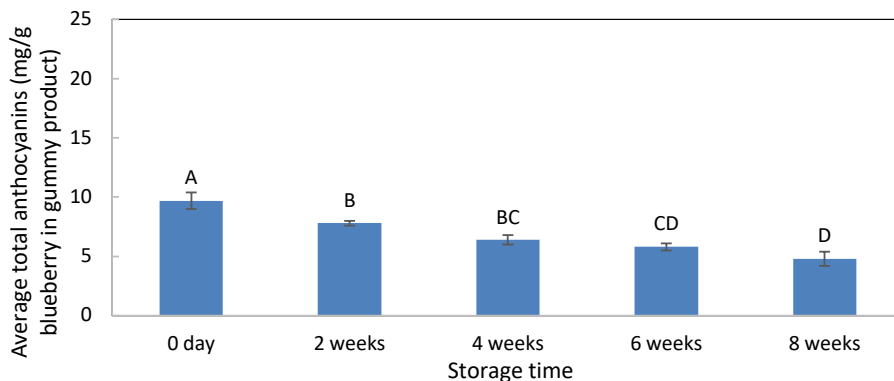


Fig. 2. Average total anthocyanins in gummy during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product ± standard error of the mean. Bars with different letters are significantly different ($P < 0.05$)

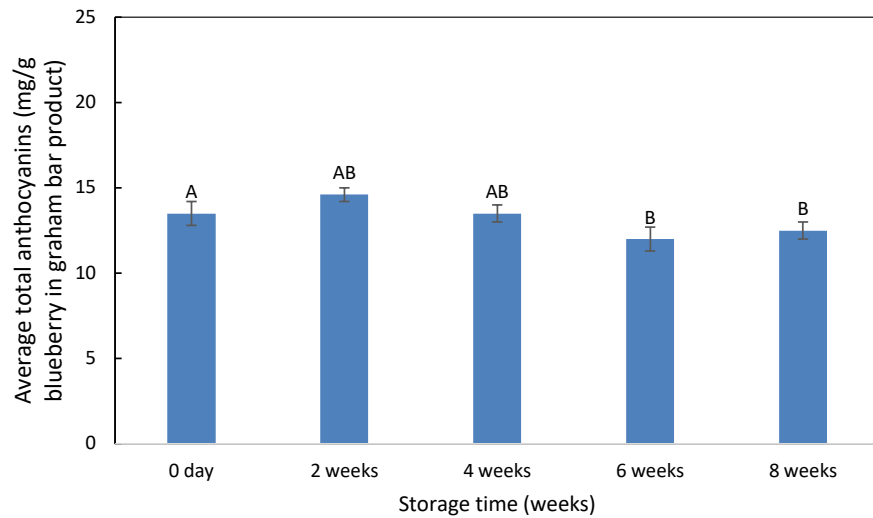


Fig. 3. Average total anthocyanins in graham bar during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$). The total anthocyanins in the graham bar remained relatively stable from day 0 to week 8. There was no significant decline in anthocyanins until week 6 and a 7% decrease in anthocyanins was measured between day 0 and week 8.

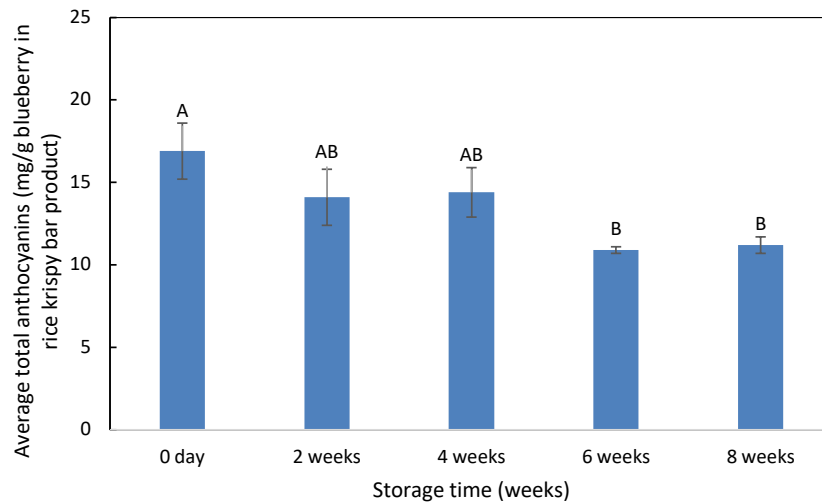


Fig. 4. Average total anthocyanins in rice krispy bar during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$).

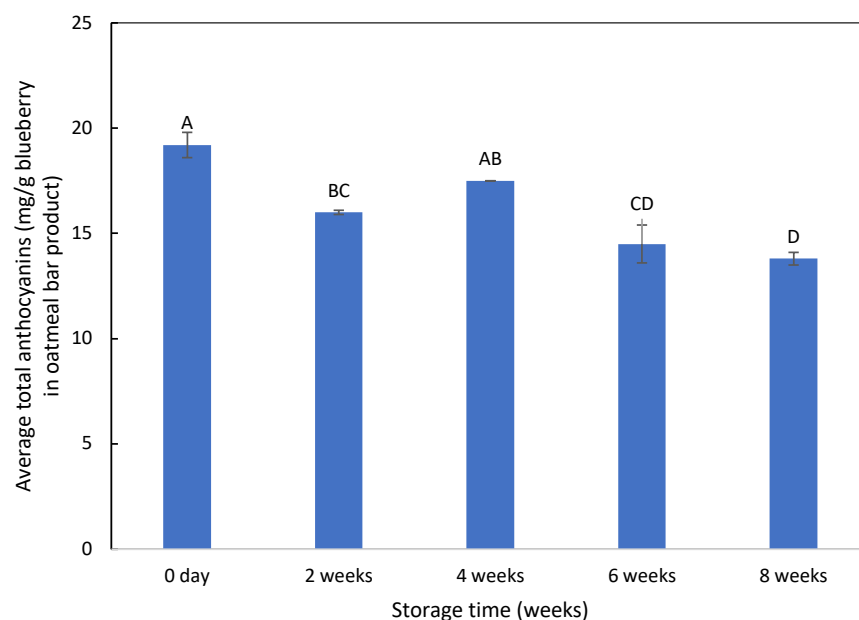


Fig. 5. Average total anthocyanins in oatmeal bar during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$).

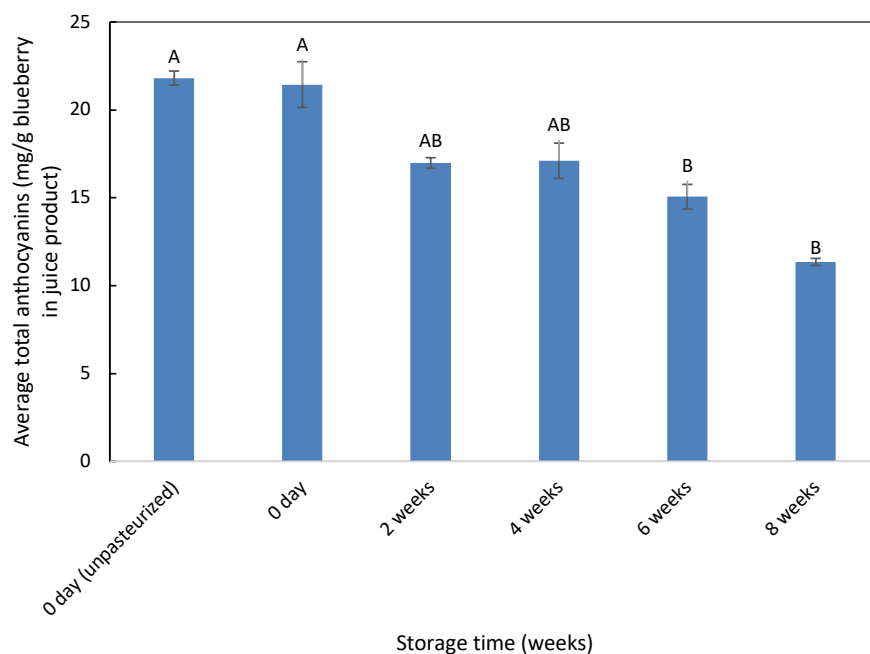


Fig. 6. Average total anthocyanins in juice during an eight-week shelf-life study. Juice from day 0 to week 8 was pasteurized. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$).

The gummy was notably the least stable of all the products analyzed, experiencing a 50% decrease in total anthocyanins during processing (Table 1) and then another 51% decrease by week 8 of the shelf-life study (Fig. 2). With the gummy only appearing to contain approximately 25% of the anthocyanins present in the blueberry powder used to formulate it, it does not seem to be as viable of an option for consistent delivery of anthocyanins compared to the other products. Additional research is needed to determine why the anthocyanins were unstable during gummy processing and storage.

The graham bar also experienced a 31% decrease in total anthocyanins during processing (Table 1). Unlike the gummy, the graham bar remained relatively stable over time with no decrease until week 6 and only a 7% decrease in total anthocyanins during the entire shelf-life study (Fig. 3). While the rice krispy bar did not experience a decrease in anthocyanins during processing (Table 1), it experienced a 34% decrease in anthocyanins during the shelf-life study (Fig. 4). The oatmeal bar also experienced a moderate 28% decrease in anthocyanins from day 0 to week 8 (Fig. 5).

While the thermal processing (pasteurization) of the juice did not decrease the total anthocyanins measured in the product (Table 1), the anthocyanins declined by 47% over 8 weeks of storage (Fig. 6). The ice pop was by

far the most stable of the products evaluated. There was no decline in total anthocyanins during processing (Table 1) or the entire shelf-life study (Fig. 7). The stability of the anthocyanins is likely due to the fact the ice pop was stored frozen rather than at ambient temperature like the other products. Freezing presumably suppressed the mechanism(s) responsible for loss of anthocyanins observed in products stored at 21 °C.

While a decrease in total anthocyanins was measured in several of the products during processing and storage, the question remains about whether the decrease was due to actual losses as a result of thermal degradation or conversion of anthocyanins into polymerized forms. Previous research found that, while some of the decreases in anthocyanins in blueberry products is due to thermal degradation during processing and storage, polymerization of anthocyanins with other anthocyanins and phenolic compounds also occurs (Brownmiller et al., 2008). Furthermore, the ability of the blueberry products to scavenge peroxy radicals (hydrophilic antioxidant capacity) was not found to decline as significantly as total anthocyanin content over storage (Brownmiller et al., 2008). This suggests these blueberry products likely do not experience as significant of a decline in bioactivity as the decrease in measured total anthocyanins (Brownmiller et al., 2008). The authors postulated that antho-

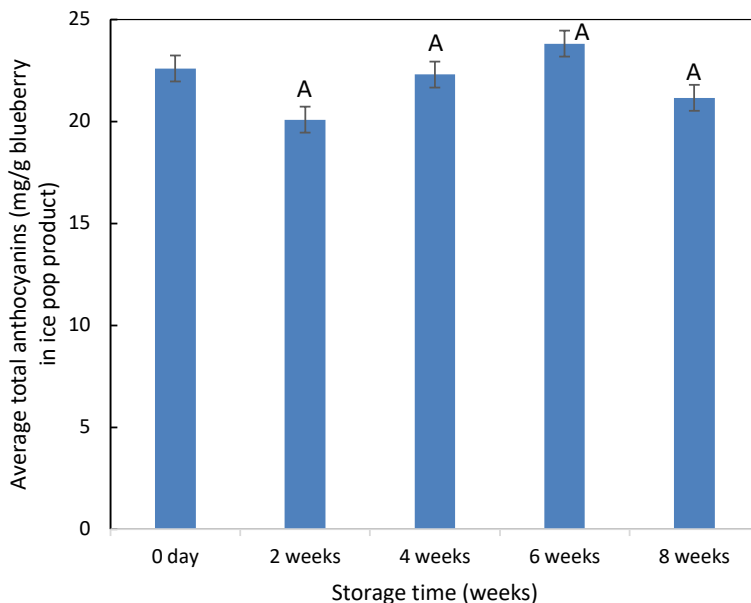


Fig. 7. Average total anthocyanins in ice pop during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. No means were significantly different ($P > 0.05$).

cyanins formed polymeric structures with procyanidins during storage, and these polymeric compounds retained potent hydrophilic antioxidant capacity (Brownmiller et al., 2008).

Conclusions

Thermal processing of gummy and graham bar products resulted in significant losses of anthocyanins (50% and 31%, respectively). An 8-week storage time also resulted in a significant decrease in anthocyanins (7% to 51%) in products stored at ambient temperature, except for the ice pop, which was stored at -20 °C. The ice pop was the best product for shelf-stability as it experienced no significant decline in total anthocyanins during thermal processing or the entire shelf-life study. Future research should be conducted to determine the differences in total anthocyanins in the products over time when they are stored under refrigeration. Additionally, polymeric color should be analyzed as this indicator has the potential to further explain the nature of the decreases in anthocyanins that were observed.

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Geospatial analysis of rickettsial species in Arkansas

Meet the Student-Author



Amy Frank

Research at a Glance

- As tick-borne illnesses become more prevalent on a state and national level, Arkansas counties are in desperate need of risk assessment for Spotted Fever group *Rickettsia*.
- A portion of the Arkansas tick population was sampled and 34% of ticks were determined to be carriers of one or more disease-causing rickettsial species.
- Several counties in Arkansas face a significant exposure risk to Spotted Fever group *Rickettsia*, and varied sample size caused an incomplete picture to be formed of others.

I was raised in Greenwood, Arkansas and have spent most of my life surrounded by animals which led me to my passion for veterinary medicine. Following graduation from the University of Arkansas, I will begin veterinary school at Oklahoma State University. I have been very active in the Bumpers Honors Student Board serving as the Director of Student Relations, Secretary, and Chair. I was also an active member of the Representing, Educating, and Promoting Scholars team for the Department of Animal Science. During my senior year, I became involved with Student Organization Outreach and Involvement Experience serving as the Director of Administration. My experiences at the University of Arkansas were made remarkable by a long list of individuals. I would specifically like to thank my thesis advisor, Dr. Ashley Dowling, who was always available to answer questions. My family (present and future) supported and encouraged me daily. Lastly, I want to thank my fiancé. His love, support, and encouragement allow me to pursue my dreams. There are no words to accurately describe the gratitude I have for all he has done for me, but I hope to show him in the years to come.



Amy is pictured here receiving the Senior of Significance distinction from Chancellor Steinmetz (left) and Brandy Cox (right), Associate Vice Chancellor and Executive Director for Arkansas Alumni Association.

Geospatial analysis of rickettsial species in Arkansas

Amy Frank* and Ashley Dowling†

Abstract

Rickettsia species are obligate intracellular, arthropod-borne bacteria with the potential to cause multiple diseases including Rocky Mountain spotted fever (RMSF). Fleas, mites, and ticks serve as vectors for *Rickettsia*, but ticks are the primary vector of interest. Rocky Mountain spotted fever and other rickettsial diseases have continued to gain importance in both human and veterinary medicine as RMSF is the most common tick-borne disease within the United States according to the Lyme and Tick-Borne Disease Research Center. A statewide citizen science project was utilized to determine the prevalence of Spotted Fever Group (SFG) *Rickettsia* in Arkansas. This project yielded results in 64 of Arkansas' 75 counties. Results were utilized to determine prevalence in each of the represented counties and then compiled into a geospatial representation of the data. It was determined that 34.32% of the ticks sampled were carriers of one or more rickettsial species. As the samples were divided by county, multiple counties were shown to have a concerning high exposure risk for SFG *Rickettsia*. There were six species of ticks represented throughout this study with *Amblyomma americanum* being the most common. There were also six species of SFG *Rickettsia* found within the samples. The small portion of ticks that underwent further analysis to determine the specific rickettsial species present indicated that *Rickettsia amblyommatis* is likely the most common SFG *Rickettsia* in Arkansas.

* Amy Frank is a May 2019 honors program graduate with a major in Animal Science with a pre-professional concentration.

† Ashley Dowling, the faculty mentor, is an associate professor in the Department of Entomology.

Introduction

Rickettsia are bacteria that live and proliferate within the cells of host organisms and have the potential to cause diseases in humans such as Rocky Mountain Spotted Fever (RMSF) (Paddock et al., 2004). Ticks operate as the primary vector of *Rickettsia* species allowing for the spread of potentially fatal diseases in humans and various animal species (Walker, 1996). Human patients endure nonspecific symptoms including fever, gastrointestinal upset, and headaches but more serious symptoms can progress such as severe myalgia, photophobia, and focal neurologic deficits (CDC, 2017a). In canines, rickettsial organisms attack vascular endothelial cells resulting in severe vasculitis, fever, ocular lesions, neurologic dysfunction, and edema (Low and Holm, 2005). Affected individuals report history of a tick bite in only 55% to 60% of cases and estimates show 60% to 75% of people are incorrectly diagnosed at the initial physician visit (Biggs et al., 2016; Herrman et al., 2014). A misdiagnosis can have severe consequences due to advanced pathological changes occurring (Raghavan et al., 2016; Gasser, 2001; Mayo Foundation, 2018).

Rickettsial organisms are typically divided into two groups, the typhus group (TG) and the spotted fever group (SFG), based primarily on distribution, pathogenicity, clinical presentation, immunological reactivity, DNA G + C mol% content and intracellular position (Fournier et al., 1998; Scarpulla et al., 2016; Ereemeeva et al., 2006). In 2010, the Council of State and Territorial Epidemiologists made a push for Rocky Mountain spotted fever (RMSF) being reported under the SFG in an attempt to facilitate more complete local and national reporting (Council of State and Territorial Epidemiologists, 2009). State health departments, including Arkansas, have recently made a push for increasing submission rates and raising awareness for tick-borne diseases (Raghavan et al., 2016).

Concern about vector-borne diseases in pets is evident by the expanding use of ectoparasite preventatives (Bowman et al., 2009). In 2003, more than half of pet owners in the United States reported using parasite preventatives (Bowman et al., 2009). The rickettsial species capable of affecting humans and canines are found to be homologous, and studies (Herrman et al., 2014) have cited canines as potential reservoirs for tick-borne diseases (Herrman et al., 2014; Warner and Marsh, 2002; Paddock et al., 2002; Kidd et al., 2006). A 40-state study found Arkansas to have the second-highest level of tick-infested canines with the six border states falling within the top ten (Raghavan et al., 2007). The risk of exposure and contraction varies in different regions with North Carolina, Oklahoma, Arkansas, Tennessee, and Missouri contributing to over 60% of RMSF cases (Atkinson et al., 2012; CDC, 2017a).

While data are readily available regarding the number of human cases involving *Rickettsia*, there is limited data demonstrating the prevalence. Rickettsial species have increased their role in animal and human health during the last few decades, which makes the need for further data apparent (Bowman et al., 2009). A geospatial analysis showing the prevalence of *Rickettsia* in Arkansas ticks may place Spotted Fever Group *Rickettsia* (SFGR) at the forefront of physicians' and veterinarians' minds. The analysis can demonstrate the areas of Arkansas that are at the greatest risk for spreading SFGR, so individuals will also be aware of the disease and the risk for contraction.

Materials and Methods

Tick Collection

In order to obtain ticks from across the state, local Arkansans were recruited to participate in the sampling process through a citizen science project. Collection kits containing five color-coded vials containing 95% ethanol and a locality recording card were distributed to all 75 Arkansas county extension offices and handed out by county extension agents. When residents collected the specimens, they were asked to record locality information or GPS coordinates. After completing the kit, citizens mailed the ticks to the Department of Entomology at the University of Arkansas or returned the tick kits to their county extension office for delivery to the University. Kits were also supplied to veterinary and medical clinics around the state. The ticks were then identified and recorded into the project database.

Molecular Methods

The DNA was extracted from individual adult tick specimens using Invitrogen™ PureLink™ Genomic DNA Mini Kits (Invitrogen, Carlsbad, Calif.) following the instructions contained therein. Nymphal ticks from the same collection event were pooled (up to five individuals per pool) and then extracted using the same Invitrogen kit. Whole ticks were extracted intact, without cutting or crushing before extraction as this was determined to not affect the extraction efficiency. The DNA extracts were screened for the presence of rickettsial species via traditional polymerase chain reaction (PCR). Fragments of the 17-kDa antigen gene were targeted using primers specific to the spotted fever and typhus group *Rickettsia* (Rr17k.1p & Rr17k.539n from Ishikura et al., 2002). Resulting products were visualized on a 1x agarose gel and a subset of positive samples was purified using Invitrogen PureLink PCR Purification kits following instructions therein. Purified samples were sent to MacroGen USA (MacroGen Corp., Rockville, Md.) for sequencing using the same PCR primers. Raw sequences were confirmed through a comparison of existing sequence data in the national sequence repository GenBank.

Data Analysis

Data analysis was conducted through Aeronautical Reconnaissance Coverage Geographic Information Systems (ArcGIS; Esri, Redlands, Calif.). This system allowed for storage, manipulation, and visualization of data with the purpose of displaying or analyzing information about places or events. The analysis was conducted in collaboration with the University of Arkansas Center for Advanced Spatial Technologies (CAST). Due to the sampling technique used with the project, prevalence is the best determinant of SFGR distribution. It helps filter out the discrepancies caused by over or under-representation of regions. The positive result prevalence for each of the 75 counties was determined using ArcGIS. The prevalence was then displayed as a geographic heat map based on obtained levels of significance. Geovisualization displays geospatial information in an interactive manner which allows for conclusions to be made and spatial patterns to be revealed.

Results and Discussion

Over the course of the study, 4676 ticks were obtained from Arkansas counties and analyzed for the presence of rickettsial pathogens (Fig. 1). Of the analyzed speci-

mens, 1605 ticks were found to be positive (Fig. 2) with the remaining 3070 ticks being negative for SFGR. Results were grouped and evaluated by county with samples being obtained from 64 of Arkansas's 75 counties. Prevalence of rickettsial species was determined using the following calculation:

$$\frac{\text{Specimens positive for SFGR}}{\text{Total specimens screened}} = \text{Prevalence of SFGR}$$

(Fig. 3). During specimen analysis, several characteristics were recorded such as the species of tick, sex, and life stage (Table 1). Prevalence by tick species in regard to the presence of SFGR was also observed and recorded (Table 2). There were 233 ticks that underwent a closer analysis to determine the specific *Rickettsia* specie(s) that was present (Table 3). The following *Rickettsia* species were found to be present in sampled ticks: *R. montanensis*, *R. amblyommatis*, *R. andeanae*, *R. bellii*, *R. rickettsii*, and *R. raoultii*.

The goal of this study was to determine the largest risk areas within Arkansas for a person or animal to become exposed to Spotted Fever Group *Rickettsia*. This is one of the only existing studies conducted utilizing geospatial analysis techniques to determine the geographic distri-

2019 TickData - 17kDA test results

- negative
- positive
- Other

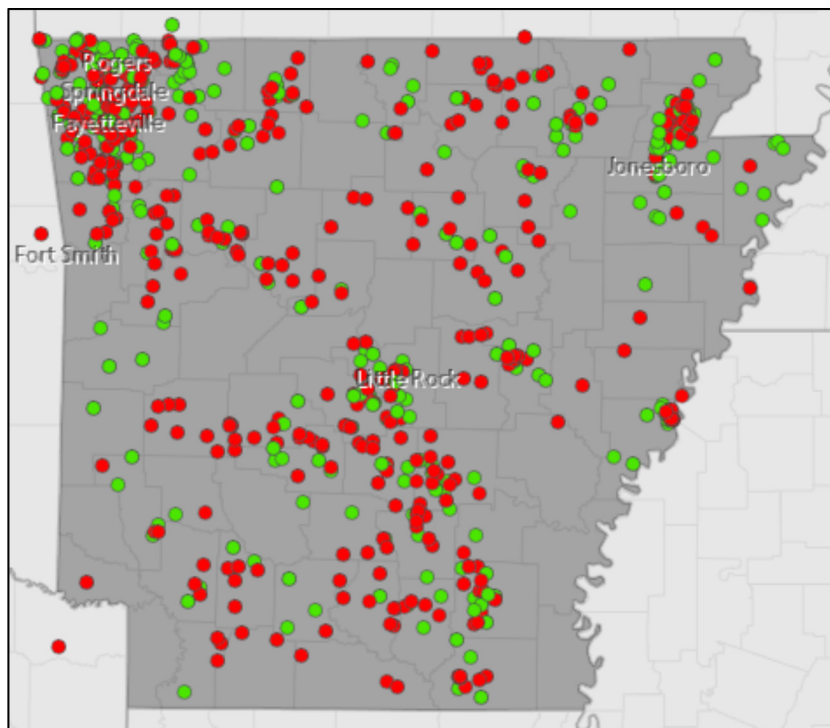


Fig. 1. Map of Arkansas displaying where tick samples were obtained based on locations given by citizen participants. Symbols represent the presence or lack of Spotted Fever Group *Rickettsia*.

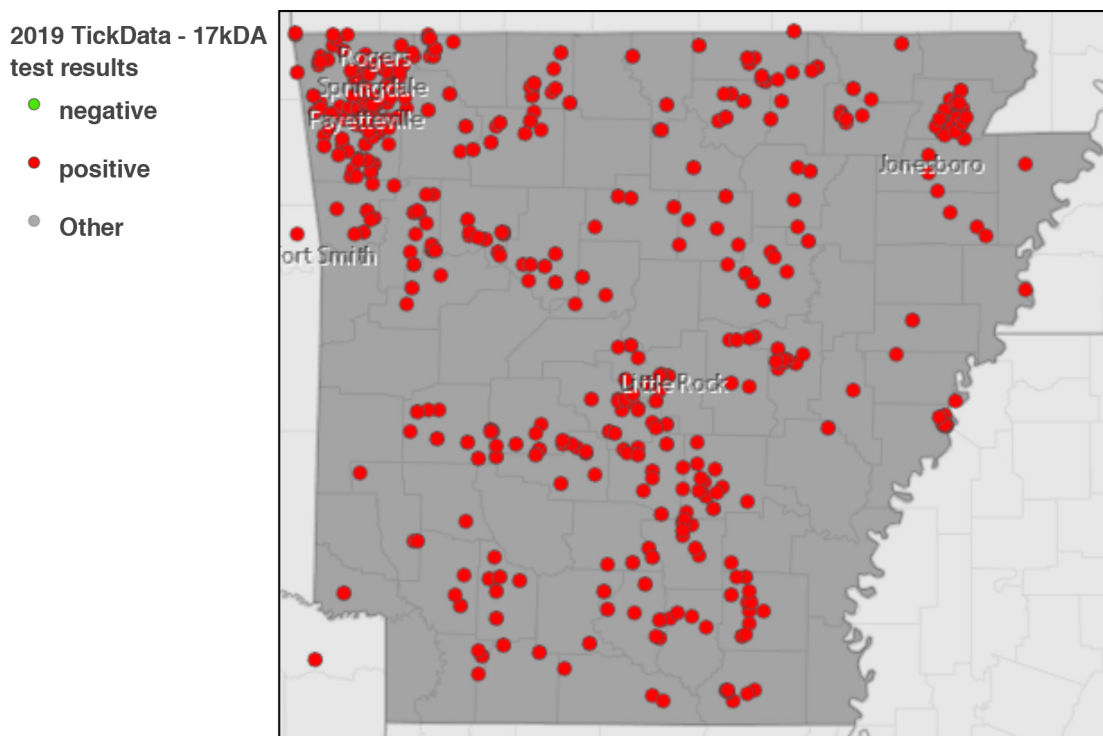


Fig. 2. Map of Arkansas displaying where tick samples were obtained that were found to be positive for the presence of Spotted Fever Group *Rickettsia*.

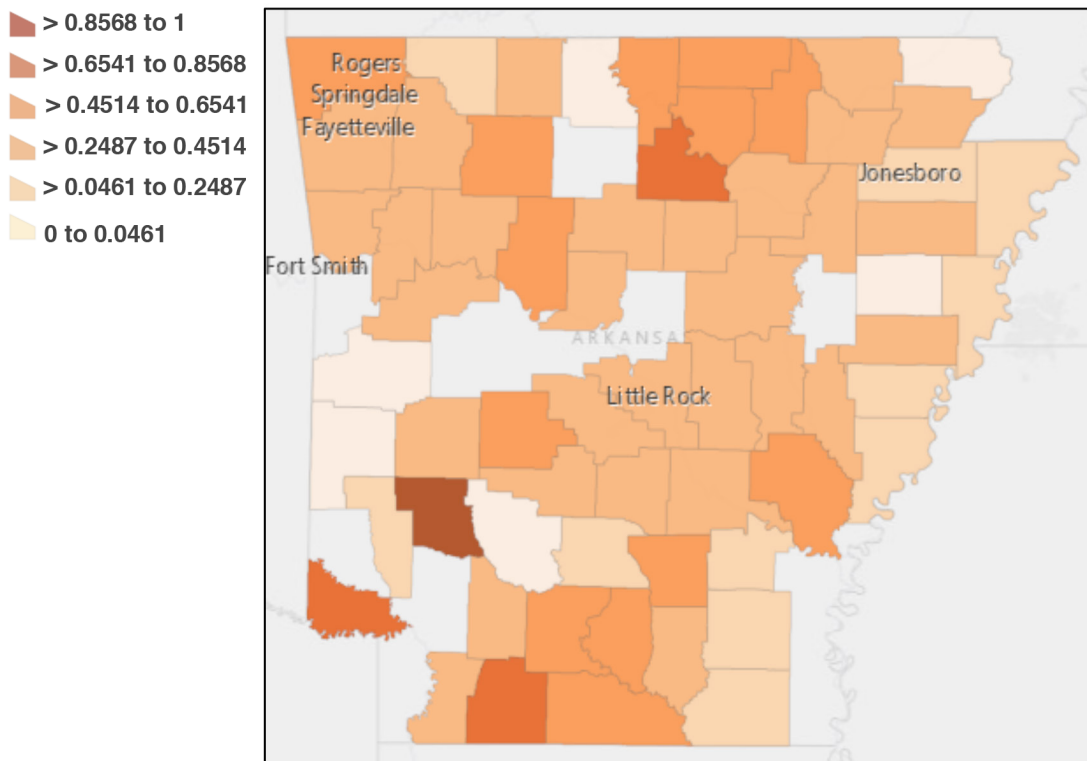


Fig. 3. Map of Arkansas displaying where tick samples were obtained that were found to be positive for the presence of Spotted Fever Group *Rickettsia*.

bution for SFGR in Arkansas, and therefore the areas that pose the greatest threat to human and animal health in the state. Samples were grouped based on which county in Arkansas they originated. This is because health departments tend to divide disease risk based on county.

When utilizing geospatial analysis, it is important to be aware of the modifiable areal unit problem (MAUP). This problem is a statistical biasing effect that occurs when samples are used to represent information for an area (Altaweel, 2018). The area is based on arbitrary boundaries, and therefore the analysis is inconsistent with real-world data. This is a common issue with health spatial statistics since statistics are typically reflecting spatial factors specific for that disease or the needs of the study (Altaweel, 2018). In this study, the prevalence of SFGR was applied to a map to demonstrate the risk of disease. The prevalence was grouped by county meaning that this study does technically fall under the criteria of the MAUP problem. To counteract the effect, more evaluation would need to be done using multiple random parameter settings. That would be irrelevant for this study, as the goal is to make the information accessible and usable to local health departments.

When looking at the display of SFGR prevalence in Arkansas (Fig. 3), it is evident that there are regions of Arkansas that face a greater risk than others. This study determined that 34.32% of the ticks sampled were carriers of SFGR. In order to determine the areas with the greatest associated risk, prevalence was utilized. This is to accommodate for the vast differences in sample size. The range in sample size was 0 to 1119 ticks.

Part of the testing process for the specimens was to determine species, sex, and life stage. The species of the tick is of interest because it is important to know which species make up the tick population of Arkansas. It is also essential to know which tick species are acting as reservoirs for SFGR. *Amblyomma americanum* was found to be the most common species making up 71% (3338 individuals) of the total ticks collected throughout this experiment. When *A. americanum* was tested for the presence of SFG *Rickettsia*, 1414 ticks demonstrated positive results. This translates to 42% of the *A. americanum* ticks tested being found to contain *Rickettsia*. Each of the six tick species found throughout this study is considered capable of transmitting rickettsial species (Lee et al., 2018; Levin et al., 2017). *Ixodes scapularis* was found to have the highest

Table 1. Species of ticks with associated common names and prevalence.

Tick species	Common name	No. ticks screened	Percentage of total ^a
<i>A. americanum</i>	Lone Star Tick	3338	71.39%
<i>A. maculatum</i>	Gulf Coast Tick	151	3.23%
<i>D. variabilis</i>	American Dog Tick	943	20.17%
<i>D. albipictus</i>	Winter Tick	1	0.02%
<i>I. scapularis</i>	Blacklegged Tick	59	1.26%
<i>R. sanguineus</i>	Brown Dog Tick	184	3.93%
Total		4676	

^a Determined with the following calculation: $\frac{\text{No. Ticks Screened}}{4676} \times 100$.

Table 2. Species of ticks with associated common names and prevalence of Spotted Fever Group *Rickettsia* (SFGR).

Tick species	Common name	No. ticks positive for SFGR	Percentage positive for SFGR ^a
<i>A. americanum</i>	Lone Star Tick	1414	42.36%
<i>A. maculatum</i>	Gulf Coast Tick	54	35.76%
<i>D. variabilis</i>	American Dog Tick	96	10.18%
<i>D. albipictus</i>	Winter Tick	0	0.00%
<i>I. scapularis</i>	Blacklegged Tick	41	69.49%
<i>R. sanguineus</i>	Brown Dog Tick	0	0.00%
Totals		1605	34.32%

^a Determined with the following calculation: $\frac{\text{No. Ticks Positive for SFGR}}{\text{No. Ticks Screened}} \times 100$.

percentage of ticks positive for SFGR. While *I. scapularis* was found to be less than 2% of the tick population, 69% were found to carry SFG *Rickettsia* species.

A small portion of the ticks sampled was randomly selected to undergo further analysis to determine the actual member of the SFG *Rickettsia* that was present. There were six *Rickettsia* species found to be present in Arkansas ticks. Interestingly, *R. raoultii* was found in 12 ticks, but only in the presence of *R. montanensis*. This is not considered uncommon as *R. raoultii* has been found to have near relationships with other members of SFGR (Li et al., 2018). Of the rickettsial pathogens found in samples, *R. montanensis* and *R. bellii* are considered of less significance as their capability to transmit disease has yet to be proven, but research has begun to suggest that *R. bellii* could eventually be found to be disease causing (Mullen and Durden, 2009; Parola et al., 2014). *R. amblyommatitis*, *R. andeanae*, *R. rickettsia*, and *R. raoultii* are known to be disease-causing members of the SFGR (Apperson et al., 2008; Delgado-de la Mora et al., 2019; Mullen and Durden, 2009). This is concerning information since the most common pathogen, *R. amblyommatitis*, was found in the most common tick, *A. americanum*.

In order to obtain a better understanding of SFGR in Arkansas, sample sizes would need to be increased for each of the counties in Arkansas. The counties that did not respond to the study or responded in low numbers should be specifically targeted. While the prevalence varied drastically from county to county, the potential to be

exposed to SFG *Rickettsia* species was abundantly clear. Other information that could be utilized in this study is the proportion of male to female ticks in the population as well as the proportion of the various life stages. This information could be useful in investigating the implication that rickettsial species have on their host. Some species of the SFG *Rickettsia* are known to have lethal effects on their tick hosts (Niebylski et al., 1999). Furthermore, time of year the specimen is obtained could be relevant information regarding when humans and animals are at most risk for being exposed to ticks.

Conclusions

Understanding the distribution of SFGR in Arkansas is essential to the veterinary and human health fields. This study showed evident regions of Arkansas that present a greater SFGR exposure risk than others. The Arkansas tick population that was sampled displayed that 34.32% of ticks are carriers of one or more rickettsial species. The aggressive human-biting tick, *A. americanum*, was the most prevalent species in the sampled population and displayed a SFGR prevalence of 42%. Concerningly *I. scapularis* was found to be a small portion of the population but showed a remarkably high SFGR prevalence. All tick species obtained throughout this project are confirmed vectors of SFGR which demonstrates why Arkansas has repeatedly been found to have one of the highest incidences of SFGR.

Table 3. Species of Spotted Fever Group *Rickettsia* (SFGR) found in ticks sampled^a.

Tick species	Known disease-causing SFGR			
	<i>R. amblyommatitis</i>	<i>R. andeanae</i>	<i>R. rickettsii</i>	<i>R. raoultii</i> ^b
<i>A. americanum</i>	206	0	0	0
<i>A. maculatum</i>	0	0	1	0
<i>D. variabilis</i>	0	1	0	12
<i>D. albipictus</i>	0	0	0	0
<i>I. scapularis</i>	0	0	0	0
<i>R. sanguineus</i>	0	0	0	0
	Not known disease-causing SFGR			
	<i>R. bellii</i>	<i>R. montanensis</i>		
<i>A. americanum</i>	0	0		
<i>A. maculatum</i>	0	0		
<i>D. variabilis</i>	1	19		
<i>D. albipictus</i>	0	0		
<i>I. scapularis</i>	0	0		
<i>R. sanguineus</i>	0	0		

^a There were a total of 233 ticks that underwent further analysis to determine which member(s) of the SFGR was present with some ticks representing more than one SFGR member.

^b This species was only found in the presence of *R. montanensis*.

Note: The ability to cause disease was based on information found in "Update on tick-borne rickettsioses around the world: A geographic approach" by Parola et al., 2014. American Society for Microbiology.

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The diversity of terrestrial mammals surrounding a waterfall at Billy Barquedier National Park

Meet the Student-Author



Kelsey Johnson

Research at a Glance

- Billy Barquedier National Park, located in the Stann Creek District of Belize has very little published research about the biodiversity in the area. Conservation efforts can be improved with more data.
- The purpose of this study was to identify terrestrial mammals using live traps, game cameras, and tracks surrounding a tourist-attraction waterfall in the park to provide a baseline list of species. Eleven different species were identified.
- This data will supplement past and future studies regarding mammal inventories both within the national park and in Belize as a whole. The data collected have been used in reports about the park and to apply for funding for further biodiversity research.

This article was written in memory of Dr. Kimberly Gray Smith: He served as an influential advisor and committee member for me and passed away on April 9, 2018.

I am from Bixby, Oklahoma and a 2019 graduate of the Pre-Professional Animal Science program. I was a member in the Pre-Vet Club, Alpha Delta Pi sorority and held two leadership positions in the Wildlife Society at the University of Arkansas. Instilled with a passion to travel, I studied abroad twice. My first trip involved research with chick dehydration on the New Horizons poultry farm in Mozambique, Africa. That opportunity lead me to an Honors thesis pilot program over the summer in Dangriga, Belize where I conducted research on the terrestrial mammals in Billy Barquedier National Park.

After my travels, I became an International Programs Office mentor for a year to encourage other students to take advantage of the amazing study abroad opportunities Bumpers College provides. I was also a member of Animal Science Representing, Educating, and Promoting Scholars (REPS) for two years. I helped REPS with events for students, faculty, and staff in the Animal Science Department and assisted with community outreach.

After graduation, I plan to attend medical school and continue traveling through medical missions abroad.

I would like to thank my colleague Mersady Redding for helping me collect data in Belize. Additionally, I want to thank my family and friends for their constant encouragement throughout my college career. I am also grateful to Peacework, Jennie Popp, Amy Farmer, Chelsea Hodge, Kimberly Smith, Jacques Hill, Jason Apple, J.D. Willson, Charles Rosenkrans, Lawton Lanier Nalley, Fred Dustan Clark, and Isabel Whitehead for their continued support. In Belize, I want to thank Peter White, Mark Faux, Fidel Brooks, Anthony Hislop, and Tanisha Cacho.



Kelsey with a Northern Climbing Rat, from the Billy Barquedier National Park.

The diversity of terrestrial mammals surrounding a waterfall at Billy Barquedier National Park

Kelsey L. Johnson^{} and Jason Apple[†]*

Abstract

Billy Barquedier is a National Park located in the Stann Creek district of Belize that contains Neotropical (the zoogeographical region which contains Central and South America) vegetation and wildlife. This study was performed to provide a baseline inventory and appearance frequency patterns of the terrestrial mammals located within Zone 1 of the park near a waterfall and to gain a greater understanding of the biodiversity and activity patterns of terrestrial mammals within the park. The methods included camera traps, small Sherman live traps, large live traps, and tracking methods. A non-random sampling method of placing camera traps and live traps on or near human-made or animal-made trails was used to identify the maximum amount of species possible within the eight-week study period. Bait including the local fruit Mamey Apple (*Pouteria sapota*) was used to attract wildlife to the study area. Based on discussions with park personnel, it was anticipated that approximately eight species would be identified within Zone 1; however, eleven different species were identified over the course of the study. The non-random sampling method introduced bias into the data. Consequently, definite conclusions about relative density and abundance of animals in the area cannot be drawn by this study alone. Statistical analysis of camera placement, length of camera placement, and time of day animal images were captured revealed that animals appeared more frequently in the central region during the first three days of image collection and during nighttime hours (2000 to 0459).

^{*} Kelsey Johnson is a May 2019 honors program graduate in the Department of Animal Science.

[†] Jason Apple, the faculty mentor, is a professor in the Department of Animal Science.

Introduction

Billy Barquedier National Park is located in the Stann Creek District of Belize. The climate in the Stann Creek district is Neotropical (zoogeographical region which contains Central and South America) and contains dense jungles with thick lush vegetation. This study took place during the onset of the wet season. The Stann Creek District has the densest river and stream systems in Belize and animals tend to congregate by water (Hakre et al., 2004). Figure 1 is a waterfall located within Zone 1. Billy Barquedier is overseen by Steadfast Tourism and Conservation Association (STACA; Dive into the Wilderness, 2017).

A biological inventory is desirable because they “are fundamental surveys that generate presence or absence of information about a species from a collection of sampling units and often serve as the first step in assessing biodiversity” and when “followed by the development of monitoring programs” are used “as a way to track changes in populations” (Gilbert et al., 2008). Camera trapping is a vital tool when collecting data for a biological inventory because it offers “researchers more reliable evidence of animal presence” (Sunarto et al., 2013).

Live trapping is a helpful method when composing a biological inventory because it yields minimum distur-

bances to population structure and density and can supplement the camera trap data by specifically identifying small rodent species that would be nearly impossible using camera data (King and Edgar, 1977). Studies have shown “trapping success of researchers and densities of small mammals varied greatly between sites” and trapping success could be low in this study (Kelly and Caro, 2003).

When a study has a short time frame, a non-random sampling method may be more beneficial because it targets “features of the landscape—such as game trails, roads, water points, and salt licks—that increase the probability of photographing one or several target species” (Cusack et al., 2015). More information regarding mammals in Billy Barquedier will be beneficial in drawing patterns of mammalian diversity and understanding their activity patterns in the area. This information can be used to guide conservation efforts within the park and throughout Belize as a whole.

Materials and Methods

Sampling Method

A non-random sampling method was used by placing camera traps and live traps on or near human-made or animal-made trails in order to identify as many species as possible in the eight-week study period.



Fig. 1. Billy Barquedier National Park, photo taken by Kelsey Johnson.

Camera Trap Methods

Moultrie (model MCG-12594 and product name M-880) cameras were set to take the photos with a one-second-trigger speed after detection, three consecutive pictures, with a five-second delay before the next trigger. “Photo series at the same camera for the same species were considered independent if 10 minutes passed with no captures of the respective species” (Kolowski and Forrester, 2017). Cameras were set up along the hiking trails either facing up or down the human-made trail, where trails created by small mammals intersected the larger human-made hiking trails, off the human trail where a game trail was located or on the ground facing small Sherman traps to capture photos of rodent species.

The information recorded for the cameras included: species, number of animals, time and date of record, camera number, longitude and latitude, and image number. The longitude and latitude were recorded using a GPS device (iPhone 6 Compass Application).

The satellite image (Fig. 2) shows the locations of the camera sets throughout this study. Location groups within the study area were joined as follows for the test: East group: camera locations 2, 3, 6 and 7; Central Group: camera locations 14, 15, 16, 19, 20, 21, and 23; and Other Group: camera locations 1, 8, 9, 10, 11, 12, 17, 18, 24, 25, 4, 5, 13, 22, 27,

and 26. All Chi-square analysis for the camera trap data was run using PROC FREQ (frequency procedure) of SAS (SAS Institute Inc., Cary, N.C.).

Live Trap Methods

Small Sherman traps (8.9 cm H, 7.6 cm W, 22.9 cm L) and larger live traps (Steel GoPlus 4.5 kg 30.5 cm H, 25.4 cm W, 81.28 cm L) were used in this study. Traps were set between 1800 and 2000 and checked the following day between 1800 and 2000 in order to minimize stress to any animal caught within the trapping period. Traps were unable to be set directly by the waterfall due to the steep rocky terrain. The small traps were set along human-made trails in areas where small rodents were expected to reside. Large traps were set along the human-made trails where there appeared to be small mammal trails intersecting. For each live trap capture, the date, time, longitude, latitude, scientific name and common name were recorded. The longitude and latitude were recorded using a GPS device (iPhone 6 Compass Application).

Bait for Camera Traps and Live Traps

The baits used included peanut butter, oats, bananas, mangos, mango jelly, Mamey apple (*Pouteria sapota*), tuna, and canned cat food.

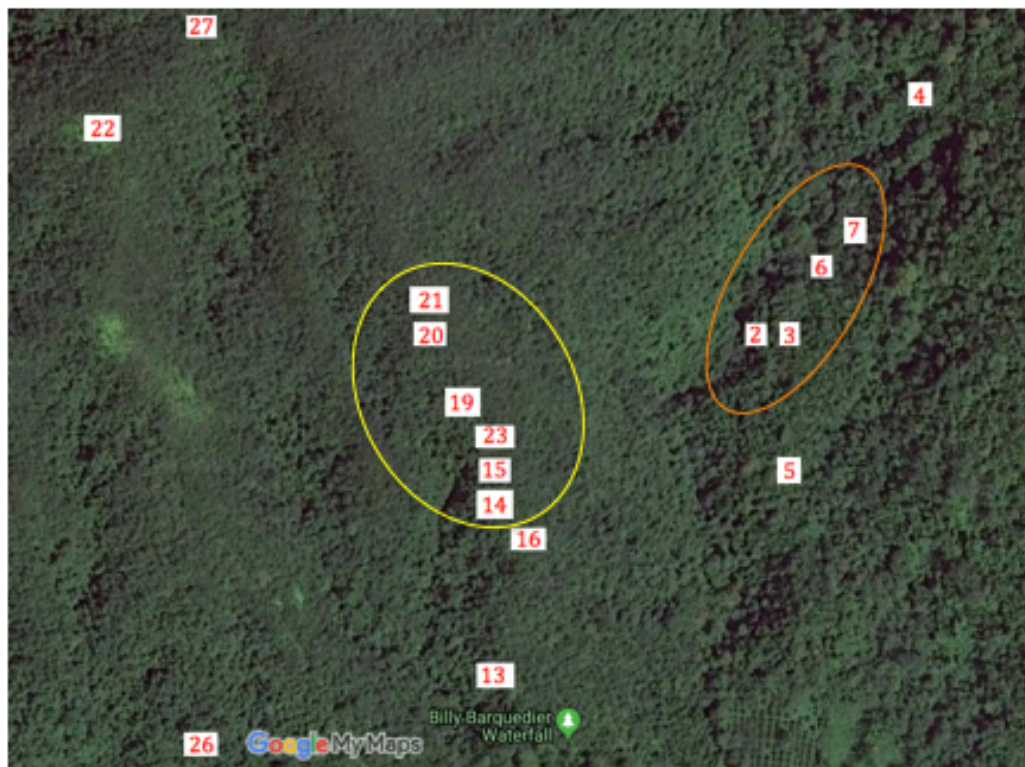


Fig. 2. Central Group camera locations circled in yellow, East Group camera locations circled in orange, and the other group includes camera locations 1, 8, 9, 10, 11, 12, 17, 18, 24, 25, 4, 5, 13, 22, 27, 26.

Tracking Methods

Knowledge of the park guides was used to aid in locating and identifying tracks made in the naturally muddy areas within Billy Barquedier. The tracks were ultimately identified using *A Field Guide to the Mammals of Central America and Southeast Mexico* (Reid, 2009) and *Neotropical Rainforest Mammals: A Field Guide* (Emmons and Feer, 1999). Once a track was identified, the common name, scientific name, date, time, latitude, and longitude were recorded.

Data Analysis

Camera location (east, central, and other) within Zone 1, length of camera placement in a particular location (ranging from 3 to 11 days of image capture), and time of the day when images were captured [dawn (DN) = 0100 to 0400; morning (AM) = 0400 to 0800; daytime (PM) = 0900 to 1600; dusk (DK) = 1700 to 2000; and nighttime (N) = 2100 to 2400] were analyzed using the chi-square option within

the frequency procedure of SAS (SAS Institute, Inc., Cary, N.C.). Although not a primary objective of this study, this information could assist future inventory surveys within the Billy Barquedier National Park.

Results and Discussion

A comprehensive summary of the results from this study is shown in Table 1.

The Northern Climbing Rat and Big-eared Climbing Rat were the only two species caught in the small Sherman live traps within Zone 1, near the end of the study period in mid to late July.

The Northern Climbing Rat (*Tylomys nudicaudus*) was captured twice, but because it was released without being marked, it was possibly the same rat captured twice. The individual appeared to be a juvenile. "No other large rats in the Northern Climbing Rat's range have a long,

Table 1. Comprehensive results.

Common name	Scientific name	Method of capture	Status	Population trend	Date last assessed	Total number of observations	Activity pattern from literature	Time of day ^a	Location ^b	Number of days camera was out ^c
Northern Climbing Rat	<i>Tylomys nudicaudus</i>	Sherman Live trap	Least Concern	Stable	8/24/16	2	Nocturnal	N/A	N/A	N/A
Big-eared Climbing Rat	<i>Ototylomys phyllotis</i>	Sherman Live trap	Least Concern	Stable	8/24/16	1	Nocturnal	N/A	N/A	N/A
Nine-banded Long-osed Armadillo	<i>Dasypus novemcinctus</i>	Camera Trap	Least Concern	Stable	10/2/13	36	Nocturnal/ Diurnal	Night (83.33%)	N/A	N/A
Striped Hog-nosed Skunk	<i>Conepatus semistriatus</i>	Camera Trap	Least Concern	Unknown	3/1/15	1	Nocturnal	N/A	N/A	N/A
Tayra	<i>Eria barbara</i>	Camera Trap	Least Concern	Declining	3/1/15	11	Diurnal/ Crepuscular	AM (45.45%) and PM (36.6%)	N/A	N/A
Collared Peccary	<i>Pecari tajacu</i>	Track Identification	Least Concern	Stable	6/24/11	N/A	Nocturnal/ Diurnal	N/A	N/A	N/A
White-nosed Coati	<i>Nasua narica</i>	Camera Trap	Least Concern	Declining	2/18/15	18	Diurnal	DN (38.89%) and AM (38.89%)	Central (100.00%)	N/A
Central American Agouti	<i>Dasyprocta punctata</i>	Camera Trap	Least Concern	Stable	6/10/16	13	Diurnal	PM (53.85%)	N/A	N/A
Paca	<i>Cuniculus paca</i>	Camera Trap	Least Concern	Stable	3/1/16	15	Nocturnal	Night (53.33%)	East (66.67%)	N/A
Ocelot	<i>Leopardus pardalis</i>	Camera Trap	Least Concern	Declining	5/11/14	4	Nocturnal/ Crepuscular	N/A	N/A	N/A
Tapir	<i>Tapirus bairdii</i>	Camera Trap ^d	Endangered	Declining	11/1/14	2	Nocturnal/ Diurnal	N/A	Other (100.00%)	N/A
Small Rodent	N/A	Camera Trap	N/A	N/A	N/A	28	N/A	Night (89.29%)	Center (75.00%)	Day 1 to 3 (53.57%)

^a Time of Day: Dawn (0500 to 0659), Morning (0700 to 1159), Afternoon (1200 to 1759), Dusk (1800 to 1959), and Night (2000 to 0459).

^b Location: Frequency of appearance of species between Center, East, and Other Locations. East group: camera locations 2, 3, 6 and 7; Central Group: camera locations 14, 15, 16, 19, 20, 21, and 23; and Other Group: camera locations 1, 8, 9, 10, 11, 12, 17, 18, 24, 25, 4, 5, 13, 22, 27, 26.

^c Number of Days: Frequency of Appearance of All Animals Between Days 1 to 3, Days 4 to 6, and Days 7 to 11.

^d Tapir tracks were also identified once.

Note: The Status, Population Trend and Date Last Accessed column information all came from the official International Union for Conservation of Nature. (IUCN) Red List website. N/A means either the method of capture did not allow this data to be collected or the data collected was not statistically significant (P -value > 0.05).

white tip to the tail” (Reid, 2009). It is typically caught in traps baited with banana or other fruits and the specimen caught in this study was caught with banana and Mamey apple. A camera was set in front of the trap and the specimen appeared to be caught on the camera (Fig. 3) eating Mamey fruit before being captured in a trap that was set on the ground under leaves (Reid, 2009).

The Big-eared Climbing Rat (*Otodylomys phyllotis*) was caught once and the trap was baited with tuna. This specimen was possibly caught on a game camera (Fig. 4)

and a trap was placed in the area it was spotted in order to try and capture it and make a positive species identification. The capture was successful with the trap placed in the ground under leaves.

A Nine-banded Long-nosed Armadillo (*Dasypus novemcinctus*) went inside one of the large traps baited with Mamey apple but unfortunately pushed its way out and defecated inside the trap. The escape appeared to be caught on one of the cameras.



Fig. 3. Northern Climbing Rat (*Tylomys nudicaudus*). This photo was taken in the field .



Fig. 4. Big-Eared Climbing Rat (*Otodylomys phyllotis*). Possible photo taken on camera trap.

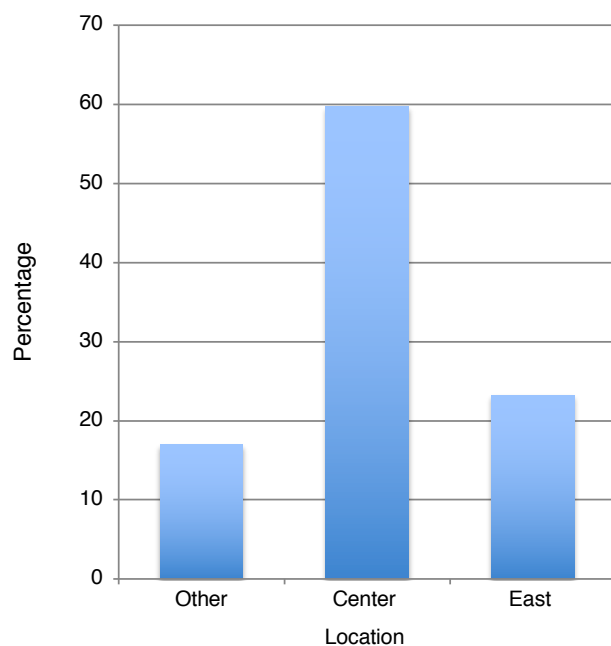


Fig. 5. Frequency of appearance of all animals between Center, East, and Other locations.

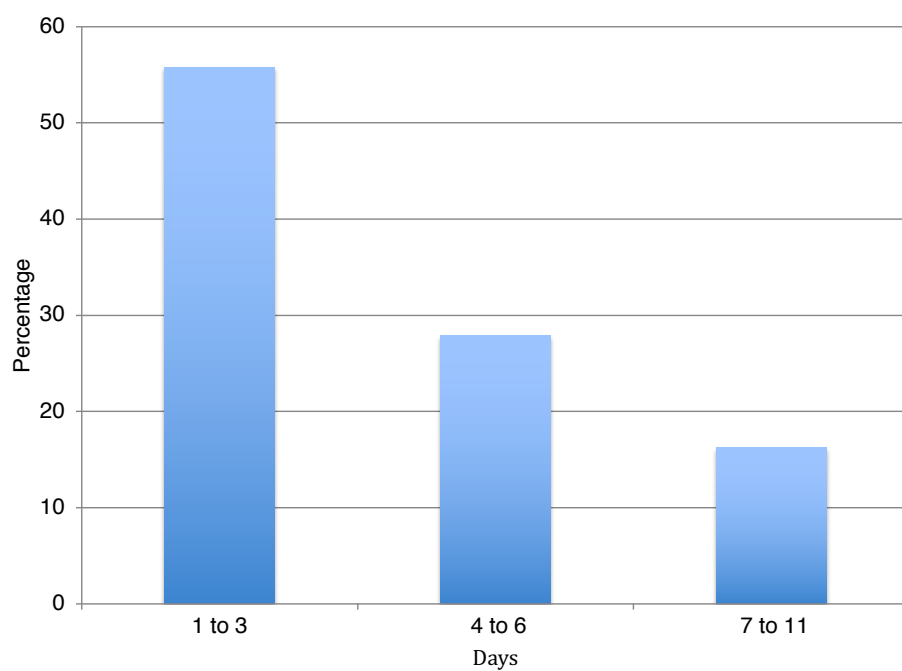


Fig. 6. Frequency of appearance of all animals between Days 1 to 3, Days 4 to 6, and Days 7 to 11.

Overall, live trapping was not widely successful in Zone 1 and no statistical tests could be conducted, but the camera trapping was promising. A total of eight species were captured on camera traps and there were two cases of human traffic captured on camera traps along Tiger Trail as well.

The group with the most frequent animal appearances was the Center Group (along Tiger Trail). A graph of these findings can be found in Fig. 5.

Comparing observation frequencies between the number of days cameras were out (Days 1 to 3, Days 4 to 6, and Days 7 to 11) revealed appearance frequencies from Day 1 to 3 were greater (Fig. 6).

When comparing activity patterns (Fig. 7) during Dawn (0500 to 0659), Morning (0700 to 1159), Afternoon (1200 to 1759), Dusk (1800 to 1959), and Night (2000 to 0459), it appears the Night category had the greatest frequency of animal observations, which leads one to believe many of the animals photographed were nocturnal.

The most prevalent species caught on camera in Zone 1 was the Nine-banded Long-nosed Armadillo (*Dasypus novemcinctus*), which was observed 36 times. The animals were not marked but showed up many times on the cameras in various locations throughout Zone 1.

The Striped Hog-nosed Skunk (*Conepatus semistriatus*) that was caught on camera at night in this study appeared to have lost almost all of its hair on its tail, which was an interesting observation that could indicate the need for

further investigation on the health of the species in the area.

The Tayra (*Eria barbara*) “may be seen singly or in pairs, occasionally groups of 3 to 4”, which is consistent with the observations in this study (Reid, 2009). One of the photos taken was of a group of three individuals eating Mamey Apple (Fig. 8).

Collared Peccary (*Pecari tajacu*) tracks were identified once and the characteristic “musty-cheese odor” was observed in the area as well (Reid, 2009).

The White-nosed Coati (*Nasua narica*) diet consists of “invertebrates found in the leaf litter and under rotting logs” and they use their “strong claws to dig, and root with the long sensitive nose”, which may be why it was attracted to cat food and tuna bait (Reid, 2009). It has been stated “males are solitary except during breeding season” and the photos in this study (Fig. 9) appeared to show one male individual traveling alone (Reid, 2009).

The Central American Agouti (*Dasyprocta punctata*) plays a vital role in the ecosystem because “when food is abundant, it carries seeds away and buries them for future use, depositing each seed in a different place. Since not all seeds are recovered this rodent is an important seed disperser for a number of tree species” (Reid, 2009). This species was observed thirteen times within Zone 1, leading one to believe that these rodents are fulfilling this important seed-dispersing role in Billy Barquedier.

There appeared to be some evidence of large cat (Jaguar or Puma) movement at the top of Tiger Trail near

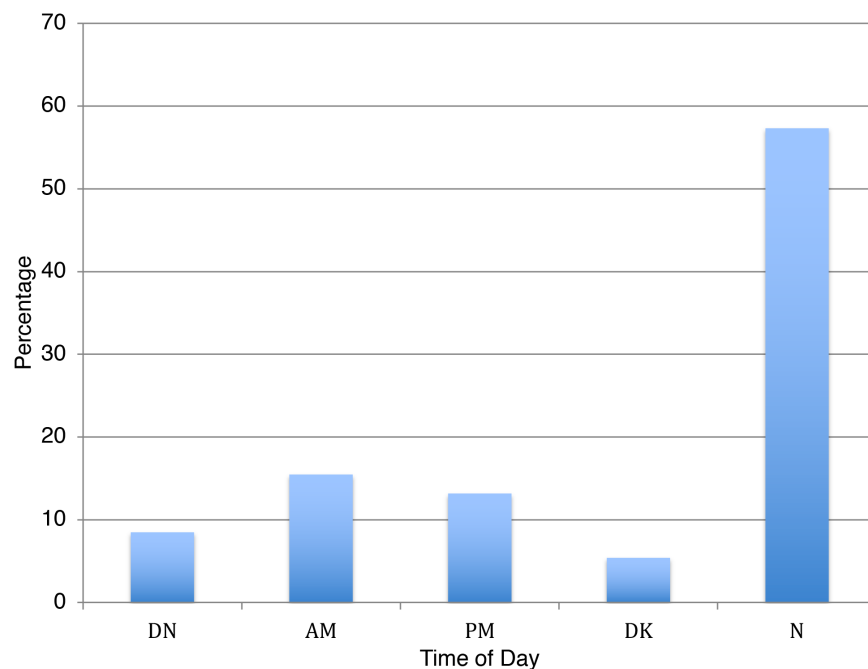


Fig. 7. Frequency of appearance of all animals between Dawn (DN), Morning (AM), Afternoon (PM), Dusk (DK), and Night (N).



Fig. 8. Tayra (*Eira Barbara*) Group of three caught on camera trap.



Fig. 9. White-nosed Coati (*Nasua narica*). Camera trap photo.

camera locations 22 and 27, due to some feces and tracks observed. There was not enough evidence for a firm positive identification, but more research in this area with camera traps could lead to a positive identification.

Of all of the baits used to attract animals, the Mamey apple appeared to be particularly successful. This “ovoid medium-large” fruit comes from the tropical tree species *Pouteria sapota* and has a “vibrant salmon-colored flesh” with a “large center pit” (Slow Food USA).

Due to the short sample period, the need to use a non-random sampling method, and the inconsistent number of days each camera was set in each location for this study, the results are subject to bias. Therefore, this species list and data is a baseline for a terrestrial mammalian inventory for Billy Barquedier National Park. Further studies must be conducted in the park to create an inventory that encompasses all of the species so further conclusions about the density and abundance within the park can be made.

In order to avoid bias in future studies and more successful species identifications, researchers “must balance the desire to maximize overall detection probability and spatial coverage given a limited number of cameras and days available for their study” (O'Connor et al., 2017). Using a randomized sampling method and camera arrays can help create this balance. Other studies suggest “the change from a single camera to even a two-camera array will likely increase detectability during the season but would reduce the number of sites being sampled by half” (O'Connor et al., 2017). With so few cameras in this study, camera arrays seem like they would be difficult to use to achieve quality data. However, the same study suggests “the increase in both survey and season detection probability over short season lengths could allow researchers to retrieve and relocate cameras, thus achieving greater spatial coverage of a landscape without sacrificing data quality” (O'Connor et al., 2017). Camera trap studies are beneficial because they show “tremendous utility in collecting wildlife data in a manner that is minimally invasive and requires reduced human labor” (O'Connor et al., 2017).

To improve studies similar to this one in the future, some alterations should be made. These alterations include: setting more camera traps in the western region of the study area, using a Garmin GPS to obtain more reliable coordinates, using camera arrays to increase the chance of successful identifications, keeping better track of what bait was used for each camera trap, and setting traps with the intention to capture specific elusive species. Fortunately, predictions that were made prior to the study were confirmed.

Agoutis (*Dasyprocta punctata*) were caught on camera within Zone 1. These rodents are a vital player in the ecosystem. They are “a caviomorph rodent” and in one study they were observed burying “13% of the seeds of

Pouteria” which is the Mamey Apple used as bait several times in this study (Brewer and Rejmánek, 1999). This means the Agouti is vital in seed dispersal for an ecosystem. This study states that small rodents are typically overlooked when considering important seed predators and dispersers in Neotropical forests even though their “great abundance and ubiquity” allows them to play a vital role in “the mechanisms that determine patterns of tree recruitment in tropical forests”, and the “results of this study support predictions by some researchers that small rodents are dominant terrestrial granivores in Neotropical forests” (Brewer and Rejmánek, 1999). Also,

since the Mamey apple (*Pouteria sapota*) was successful bait in this study, future researchers in Belize should consider this local fruit when trying to attract wildlife.

In all, the data collected in this study could supplement mammal inventory in the journal article “Inventorying mammals at multiple sites in Maya mountains” (Caro et al., 2001).

Conclusions

The initial hypothesis in this study was with the time frame given (approximately eight weeks) at least eight species would be identified using camera traps, live traps, visual identification, and track identification. The results revealed a total of eleven different species identified within Zone 1 of the Billy Barquedier National park, which exceeded the expectation of eight, or fewer, species. Also, chi-square analysis of image collection parameters indicated that animals appeared more frequently in the central region of the study site, during the first three days the cameras were set out, and during the nighttime hours (2000 to 0459). Eleven species for an eight-week study period is considered a success based on previous studies performed in the neotropics. A longer study period, more randomized camera placements, consistent durations cameras are set out before being switched (between three to seven days after a camera is set it should be re-baited or relocated to maximize species detection), and more randomized live trap placements may yield results that capture the more elusive species (for example the Jaguar, Puma, and more small rodent species) present in Billy Barquedier National park. The location of Zone 1 might have also played a role in seeing less elusive species due to human traffic (humans were spotted twice on camera traps). The pictures and data collected in this study were given to the overseers of Billy Barquedier National Park (STACA) and have been used in reports about the park and to apply for funding for further biodiversity research in the park. Overall, the data produced by this study can supplement other terrestrial mammal species inventory

studies that have been done in the past and other biodiversity research that will be performed in the future.

Acknowledgements

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Identifying Arkansas Food Desert Blocks Suitable for a Peer-to-Peer Modeled Food Redistribution Program

Meet the Student-Author



Emily King

In May of 2019, I graduated from the University of Arkansas Dale Bumpers College of Agricultural, Food and Life Sciences with degrees in Agricultural Business & Marketing Management and Food Technology. This research was conducted in partial fulfillment of the requirements for the University of Arkansas Bumpers College Honors Program. During my time at the University of Arkansas, I have been involved in Phi Mu Sorority, the Arkansas Union Advisory Committee, Food Science Club, the Volunteer Action Center's literacy and food assistance programs, and served as a Student Ambassador. I also served as a Bumpers College Honors Mentor and University of Arkansas Orientation Mentor. My most enjoyable experience at the University of Arkansas was my international study abroad in Greece where I studied agriculture and food sustainability from the Greek and European Union perspective. During the summer between my junior and senior year, I interned with ConAgra Brands in Omaha, Nebraska as a part of the Product Lifecycle Management team. While at the U of A I was named the Dale Bumpers College Presidential Scholar, Outstanding Student, and Alumni Association Senior of Significance and Razorback Classic. After graduation, I will pursue my juris doctorate at Michigan State University College of Law. I am thankful to Dr. Jennie Popp for her assistance throughout this research project as well as to my parents, Marlon and Michelle King for their guidance throughout my life.

Research at a Glance

- This study identified food deserts in the state of Arkansas, which are areas that have large proportions of households with low incomes, inadequate access to transportation, and a limited number of healthy food retailers.
- An analysis was conducted using population, internet access, vulnerable communities, and vehicle availability as criteria to identify which of the Arkansas food deserts are best suited for a program that redistributes food in a peer-to-peer way.
- From the results of this study, it is recommended that Pulaski County be targeted for a food redistribution program that provides residents with an online platform for selling unused or unwanted food items.
- This study can be used to analyze food desert locations in Arkansas for redistribution programs and serve as a baseline for future studies pertaining to the implementation of peer-to-peer economic models.



Emily in front of the Parthenon in Athens, Greece during her study abroad experience.

Identifying Arkansas Food Desert Blocks Suitable for a Peer-to-Peer Modeled Food Redistribution Program

Emily King^{}, Jennie Popp[†], Michael Thomsen[§],
Di Fang[‡], and Alvaro Durand-Morat[¶]*

Abstract

Nearly 10% of Americans reside in low-income urban food deserts, which are low-income areas that lack access to affordable and nutritious foods. Food deserts in Arkansas contribute to a food insecurity rate above the national average, making it one of the most food-insecure states in the country. Increased internet usage and consumer interest in sharing-based companies contribute to the idea of a sharing, or peer-to-peer (P2P) style food redistribution program. The objective of this study is to identify which of the 186,211 census blocks in the state of Arkansas are food deserts and best suited for and in the most need, based on an identified set of criteria, of a P2P food redistribution program. A multi-criteria decision analysis was conducted using population, internet access, vulnerable communities, and vehicle availability as criteria. Results suggest that based upon the proximity of priority areas, transportation access, ethnic/racial diversity, and the number of possible collection locations, Pulaski County should be targeted for a P2P food redistribution pilot program.

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‡ Di Fang, assistant professor, Department of Agricultural Economics and Agribusiness.

¶ Alvaro Durand-Morat, assistant professor, Department of Agricultural Economics and Agribusiness.

Introduction

A large number of food deserts in Arkansas make it one of the most food insecure states in the country (USDA-ERS, 2017b). Food deserts are regions of the country that “often feature large proportions of households with low incomes, inadequate access to transportation, and a limited number of food retailers providing fresh produce and healthy groceries for affordable prices” (USDA-ERS, 2017a).

A peer-to-peer (P2P) economic model could serve as a possible solution to the problem of urban food deserts, which make up 75% of total food deserts (National Coalition for the Homeless, 2011). A P2P economy is a model where individuals interact to buy or sell goods and services directly to one another, without an intermediary or company. Airbnb and Uber are examples of successful P2P organizations. Food sharing has become more common in cities and often focuses on redistribution of surplus food (Gaspard, 2018). Redistributing surplus food through a P2P system can positively impact food deserts and reduce the big problem of food waste. The objective of this study was to identify food desert census blocks in the state of Arkansas that are best suited for and in the most need, based on an identified set of criteria, of a P2P food redistribution program.

Materials and Methods

The assessment was conducted based on multi-criteria analysis and the methodology was inspired by the steps set forth by Haque (2016).

All food desert blocks in the state of Arkansas were identified based on income level and access to nutritious foods. Poverty and median income data from the U.S Census were used to determine whether each block's poverty rate was 20% or greater or each block's median family income was less than or equal to 80% of the state-wide family income (USDA-ERS, 2017a). Data regarding grocery store and supermarket locations, typical suppliers of nutritious foods, from Burgener and Thomsen (2018) were used to determine low access. Data arrangement and mapping were completed using RStudio (RStudio®, Boston, Mass.).

Criteria for selection were based on population, internet access, vulnerable households, and vehicle access within food desert block groups. Within this study, alternatives, or block groups, were initially scored on an interval scale for internet access, vulnerable communities, and vehicle access criteria. Population was the only criterion that was not scored. Data classification by quantiles was used to classify data into a specific number of categories with an equal number of units in each category.

ry. One thousand quantiles were calculated and used for each criterion. The quantiles ranged from 0.1th to 100th, each with a corresponding value. The census blocks were scored 1 to 1000 depending on which quantile their criteria value fell into. The rationale for each included criterion is briefly presented below.

Population

A P2P food sharing program provides users with perishable goods that cannot necessarily be shipped in 2 to 3 business days. Therefore, buyers and sellers must be in proximity to one another. To follow this idea, block groups with higher population density, or in other words more urban, are preferred for implementation of this program.

Internet Access

Peer-to-peer markets rely on sharing goods and services through new information systems on the internet (Hamari et al., 2016). In order for a P2P food redistribution program to work within a food desert, the residents need access to the internet through a subscription or other means. The percentage of households with internet access was determined using the 2013–2017 American Community Survey 5 Year Data Table B28002 Presence and Types of internet Subscriptions in Household. These data were calculated at the census tract level because the information is not collected at the census block group level. Tracts with a high percentage of households with internet access are likely highly compatible with the P2P program.

Children Under 18

The percentage of residents in each food desert block group under the age of 18 was determined using the 2013–2017 American Community Survey 5 Year Data Table B01001 Sex by Age. These data were calculated at the census block group level. Block groups with a high proportion of children are likely at a higher need for the P2P program.

Minority Population

Poverty also is an indicator of food deserts (USDA-ERS, 2017a). In Arkansas, Black and Hispanic households are roughly two times more likely to live in poverty, elevating their risk of food insecurity and residing within a food desert (2017 American Community Survey 1 Year data. Tables B17001A, B17001B, and B17001I; Bred for the World, 2018). The percentage of residents in each food desert block group that are either Black and/or Hispanic was determined using the 2013–2017 American Community Survey 5 Year Data Table B03002 Hispanic or Latino Origin by Race. These data are calculated at the

census block group level. Block groups with a high proportion of Black and/or Hispanic residents are likely at a higher need for the P2P program.

Vehicle Availability

Food desert residents without access or ownership of a vehicle may be at a higher risk for food insecurity as a result of limited full-service food retailer access or high food prices at local food retailers (Fitzpatrick and Ver Ploeg, 2010). The percentage of residents in each food desert block group without access to a vehicle was determined using the 2013–2017 American Community Survey 5 Year Data Table B25045 Tenure by Vehicles Available by Age of Householder. These data are calculated at the census block group level. Block groups with a high percentage of residents who do not have an available vehicle are likely in high need of the P2P program.

Each criterion was given weight. The criterion, with the exception of population, have an impact range of 1000, meaning the maximum score for each criterion is 1000.

To value certain criteria more than others, the four criteria were weighted according to importance. Criteria with heavier weights are more important in determining the location most suitable for P2P activity. Based on the above-mentioned literature as well as Gal-Or (2017), Wright et al. (2016), and Feeding America, (2018), criteria were placed in this order of importance and assigned the following weights: internet (32%), Children Under 18 (26%), Minority Population (22%), and vehicle availability (20%).

Each criterion was scored. Initial scores (1–1000) were multiplied by the corresponding criteria weight. Final scores were totaled to provide a single score for each block group. Each block group was able to score up to 1000 total points. Urban food desert blocks that scored 75% or more of the possible points (750 or more points) were identified as priority blocks. To determine if there is one specific area of the state that is far more in need of the pilot program, the top five (or less) priority areas were identified. Using 900 points (90%) as the determinant was able to provide less than five high priority block areas.

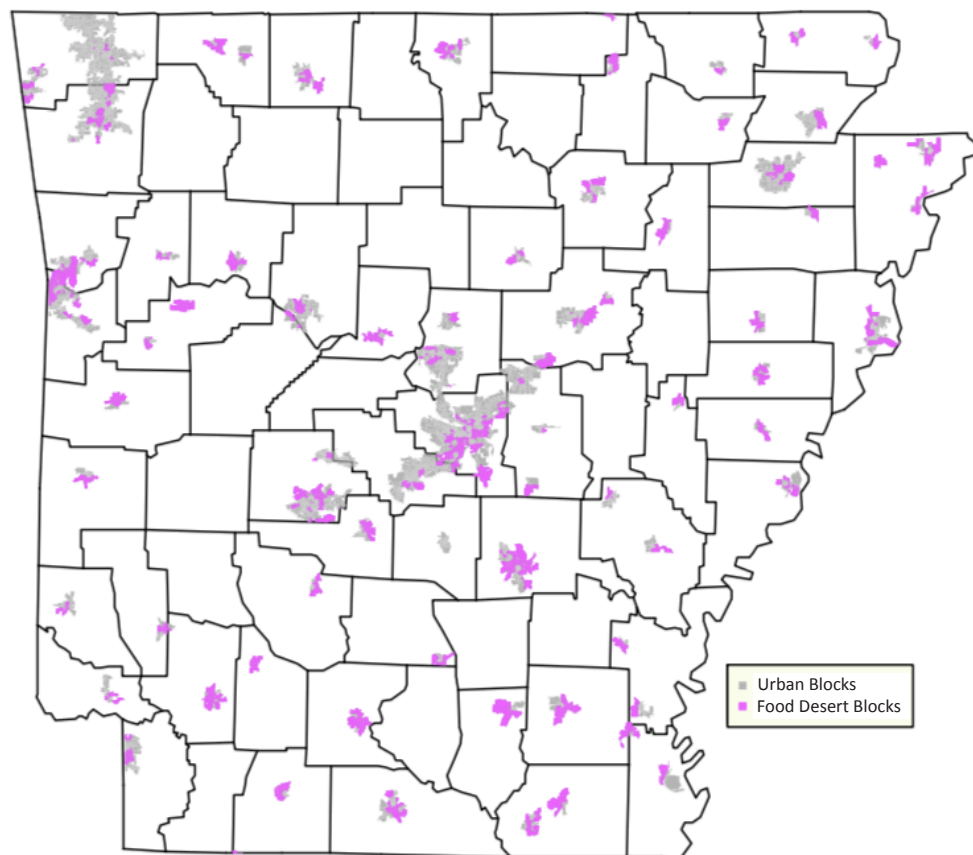


Fig. 1. Identified food desert blocks layered on top of urban blocks.
Source: King 2019, using data from 2010 U.S Census, Burgener and Thomsen 2018.

Results and Discussion

To begin, 26,700 food desert blocks in Arkansas were identified, and they appeared in every county. For the implementation of the proposed program, census blocks with higher population density, or more urban areas were preferred. Using the U.S. Census Bureau's census-designated places, Fig. 1 was derived and shows the identified urban food deserts layered on top of the urban blocks. Figure 1 shows 57,925 urban blocks, as defined in this study. As expected, cities with over 50,000 residents such as Little Rock, Fayetteville, Springdale, and Jonesboro were included in the urban block mapping. After locating the urban food desert blocks, four further criteria; internet access, child population, minority population, and vehicle availability were used to score and weight the varying block groups. Urban food desert blocks that scored 75% or more of the possible points (750 or more points) were identified as priority blocks.

In Fig. 2, there were areas including Pulaski and Garland County that have multiple priority areas in proxim-

ity to one another. The high number of priority areas in and around Little Rock in Pulaski County, as shown in Fig. 3, makes it of high interest. There are roughly 14 priority areas in Pulaski County. Given their proximity and likelihood to reach a lot of people, there are three specific large priority areas, circled in Fig. 3.

There are variables that were not included in the scope of this analysis, but still play a role in the success of the P2P program. These variables include transportation access, ethnic/racial diversity, and the number of possible collection locations. If food is being transported from surrounding cities or states, there needs to be an efficient way to access food desert areas. Little Rock possesses this ability because it is located at the intersection of two major highways, Interstate 30 and Interstate 40. This location makes the transportation of redistributed food easier than it would be if the program was placed in an area such as Jonesboro or Hot Springs.

As previously mentioned, in Arkansas, Black and Hispanic households are more likely to live in poverty, elevating their risk of food insecurity and residing within

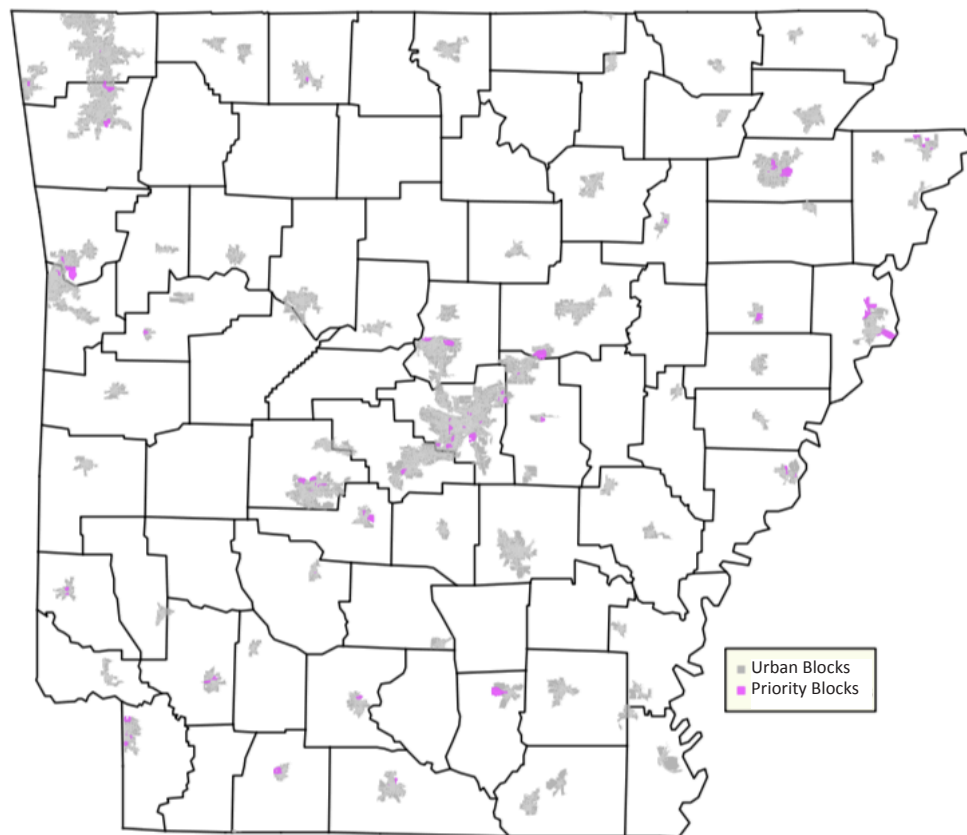


Fig. 2. Identified priority blocks, those with 750 or more points layered on top of urban blocks
Source: King 2019, using data from 2010 U.S. Census and 2013–2017 American Community Survey 5 Year Data Tables.

a food desert (2017 American Community Survey 1 Year data. Tables B17001A, B17001B, and B17001I; Bread for the World, 2018). Pulaski County and Little Rock are ethnically and racially diverse, which further identifies them as good locations for P2P activity.

Though collection and distribution location data were not included in this study, it is assumed there are numerous places in Pulaski County to choose from. Pulaski County is the most populated county in Arkansas and Little Rock is the most populated city. It is well known that larger cities and counties have more establishments, therefore, finding a location for a P2P program collection and distribution point would be easier.

As shown in this study, there are multiple reasons to target Pulaski County for a P2P food redistribution program. First, Pulaski County possesses three large priority areas with high levels of internet access in proximity. These priority areas are just under two miles apart, therefore, placing a P2P activity hub in between the top area and the middle area and between the middle area and bottom area would provide food access less than one mile from residents. This could transition these priority areas away from food desert classifications. Using the U.S. Census Bureau's population density for Little Rock (1623.5 people per square mile), it is estimated P2P activity in these areas could service around 7500 residents (Census.gov: QuickFacts: Little Rock city, Arkansas; Pulaski Coun-

ty, Arkansas; Arkansas, 2018). Next, Pulaski County is located at the intersection of two major highways, making it easy to access by transportation. Pulaski County is more ethnically and racially diverse than the state of Arkansas as a whole indicating it is in more need of a food access program. Finally, Pulaski County has a high population and many potential locations for collection and distribution sites. Based on the results of this study, it is recommended that Pulaski County be amongst the first to be targeted for a P2P food redistribution program pilot.

Should additional studies further examine issues related to a P2P modeled food redistribution program in Arkansas, the following recommendations are made. First, the identification of collection and distribution points are needed. This study simply identifies where in the state of Arkansas is most suitable and in the most need of a food redistribution program, but it does not pinpoint specific locations for the program's primary hub. Data regarding the locations of farmers' markets, churches, and pantries were not included in this study. Within the priority blocks and the clusters of priority blocks, it would be beneficial to identify farmers' markets, food pantries, churches, or other community facilities to serve as collection and distribution points. After finding these locations it would be helpful to then determine the number of food desert residents that could be reached and impacted by the program.

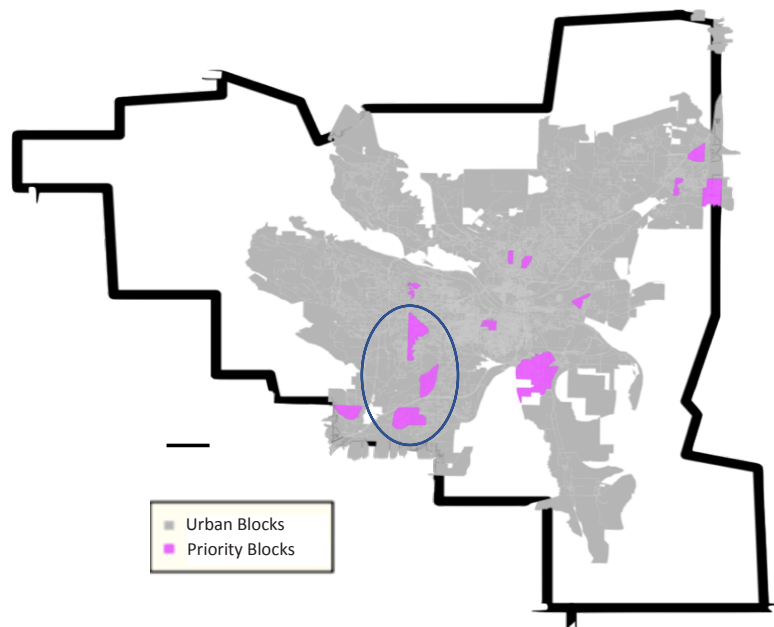


Fig. 3. Identified priority blocks in Pulaski County, those with 750 or more points layered on top of urban blocks.
Source: King 2019, using data from 2010 U.S. Census and 2013–2017 American Community Survey 5 Year Data Tables.

Secondly, government funding may play an important role in launching a program of this size, especially if SNAP benefits are to be used via the app or website. For program funding and policy implementation, it is important to show if this program in the selected location can benefit minorities and SNAP beneficiaries. This study takes a broad approach in determining priority areas which include the minority population, but not the number of SNAP beneficiaries. Within the priority blocks and the clusters of priority blocks, it would be beneficial to identify where large populations of minorities are located just as was done in the map of internet access in Pulaski County. It would also be beneficial to show the number of SNAP beneficiaries in the priority blocks to signal if there is a need for P2P accessible SNAP benefits.

This study does not determine whether residents of these areas would enjoy or participate in the outlined P2P program. After areas and collection/distribution points are identified and before the program is implemented, it would be important to understand if residents would be interested in joining a P2P style system and what obstacles they foresee. Allowing residents to play a role in designing the final program can help ensure they participate in it after implementation.

Finally, this study does not conduct a sensitivity analysis for the criteria weights. This is a limitation because different percentages may better identify priority areas. In future studies, conducting a sensitivity analysis may be useful.

Conclusions

This study may be used to 1) help analyze food desert locations for P2P activity implementation in Arkansas, and 2) expand the study to include other states and food deserts in the U.S. Finally, this study could serve as a baseline to a future study that examines the location of P2P food redistribution collection points and the number of consumers they could reach.

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Evaluating rice straw as a substitute for barley straw in inhibiting algal growth in farm ponds

Meet the Student-Author



Jacob Maris

Research at a Glance

- Algal blooms can harm aquatic ecosystems and have become more common and severe due to nutrient pollution. Conventional mechanical and chemical methods of algal population control are inefficient and can harm other aquatic organisms.
- Aerobically decomposing barley straw has been shown to inhibit the growth of algal populations. Barley is not a common crop in Arkansas, but other cereal grain straws may release similar chemicals. Rice straw represents a possible eco-friendly, locally sourced form of algal control.
- Based on the results of this study, neither barley straw nor rice straw was effective at algal growth inhibition compared to the control.

I am from Little Rock, Arkansas and graduated from Little Rock Central High School in 2015. In May of 2019, I graduated magna cum laude from the Dale Bumpers College of Agriculture, Food, and Life Science with a degree in Environmental, Soil, and Water Science and a minor in Agricultural Business. Funding was generously provided by the Honors College and Bumpers College to conduct this research and present the results in the undergraduate oral research competition at the ASA, CSSA, SSSA annual conference in Baltimore, Maryland.

I developed a love for both the Razorbacks and the outdoors at a young age. As I learned about our environment and the impact humans have on it in middle and high school, I knew I wanted to make a career out of minimizing that impact. Over the past four years, I have been able to combine these two passions by attending the University of Arkansas. While an undergraduate, I had the opportunity to participate in study abroad programs in Belgium and New Zealand.

Thank you to Dr. Brad Austin for his help in sample analysis and to Dr. Ben Runkle and Dr. Trent Roberts for providing rice straw. I would also like to thank Jody Davis, Brian Austin, Greg Cheshier, Jean Hammack, and LaJoyce Duncan for allowing me to use their ponds in my study.



Jacob analyzes nitrate-N concentrations of pond water samples in the laboratory.

Evaluating rice straw as a substitute for barley straw in inhibiting algal growth in farm ponds

Jacob Maris^{*}, Mary Savin[†], and Lisa Wood[§]

Abstract

Algal blooms disrupt aquatic ecosystems and are more common in lakes, ponds, and rivers during the summer months due to nutrient pollution. Livestock production can contribute increased quantities of nutrients to water bodies from runoff of manure. Commonly used mechanical and chemical control methods may have limited success because algae are small and propagate quickly. Barley (*Hordeum vulgare*) straw has been shown to inhibit the growth of algae as the straw decomposes aerobically in ponds. Therefore, barley represents a natural option for algal biomass control. However, the small amount of barley production in Arkansas limits the availability of barley straw as a solution to control algal blooms locally. Other cereal grain straws may produce similar inhibitory effects during decomposition. Rice (*Oryza sativa*) is produced in large quantities in Arkansas, making rice straw a locally sourced straw product. The objective of this research was to determine the efficacy of using rice compared to barley straw to inhibit algal growth in freshwater ponds. Data were collected from nine farm ponds, three treated with rice straw, three treated with barley straw, and three without amendment to serve as the experimental control. Dissolved oxygen, pH, nitrate-nitrogen (NO_3^- -N), dissolved phosphorus (P), temperature, and turbidity were measured for 14 weeks from 12 June to 17 September 2018. Algal biomass was measured as chlorophyll-a concentration to evaluate treatment effectiveness over time. Dissolved oxygen was significantly influenced by the main effects of treatment and time. The NO_3^- -N concentration in ponds treated with rice straw was significantly greater than the control and barley treatment. Chlorophyll-a concentrations were variable, and there were no consistent trends through time within a treatment. More research under controlled conditions to understand impacts of abiotic conditions, microbial and algal community compositions, and mode of action of algal inhibition is required before cereal straw can be a reliable, locally sourced method of algal control in farm ponds.

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Introduction

Algae are present in almost every aquatic ecosystem, play a key ecological role through photosynthesis, and serve as a food source for higher trophic levels. Phytoplankton are free-floating algae that live in the upper layer of stratified ponds and lakes and can grow to large numbers forming algal blooms. These algal blooms, or elevated densities of algal populations, compromise ecosystem health. Increased nutrient concentrations from human activities, such as fertilizer use and livestock production, contribute to more frequent algal blooms (Islami and Filizadeh, 2011). The increase in algal abundance can turn the water color, commonly green in freshwater, and can cause a foul odor. Additionally, dissolved oxygen becomes limited as the algae die and decompose (Kannan and Lenca, 2012). Blue-green algae, while grouped with algae, are photosynthetic bacteria called cyanobacteria (Kannan and Lenca, 2012). Blue-green algae can turn the water green, produce a foul odor, and release cyanotoxins that may be harmful to humans and animals. Attempts to control algae are rarely successful because algae are small and reproduce quickly. Mechanical removal is inefficient and must be repeated periodically, while treatment with chemical algicides can harm non-target organisms (Swistock, 2017).

Barley (*Hordeum vulgare*) straw can be used as an alternative method of algal control. As barley straw decomposes aerobically, it releases a chemical, or combination of chemicals, that inhibits the growth of green algae and cyanobacteria without harming other aquatic life (Islami and Filizadeh, 2011). While the precise inhibitory chemical is not known, it has been hypothesized that weak peroxides and oxidized polyphenols are responsible for algal growth inhibition (Islami and Filizadeh, 2011). Straw must be placed in ponds 2 to 8 weeks before the algal growing season, depending on water temperature, to give the straw time to begin decomposing (Lembi, 2002). Maximum toxicity to blue-green algae occurs after one month of decomposition and declines over the following months until decomposition is complete (Rice et al., 1980). Decomposition of the barley straw may decrease dissolved oxygen, but the lack of competition for light from algae allows more photosynthesis from higher-order plants (Newman, 2004).

While barley straw has the potential to be an environmentally “clean” form of algal control, there are some concerns regarding the adoption of barley straw for algal control. Barley straw acts as an algistat, rather than an algicide, such that barley straw does not kill existing algal cells but prevents the growth of more algae. Because the Environmental Protection Agency has not certified barley as an algistat, barley straw can be marketed legally only as a home remedy for preventing algal growth (Lembi, 2002). A lo-

gistical challenge to using barley straw in Arkansas is that barley is not a commonly cultivated crop (USDA, 2018). Barley production in the United States is concentrated in the northern midwestern and northwestern states, such as North Dakota, Montana, and Washington, rendering barley straw unavailable to much of the country (Guercio, 2018).

Arkansas is the largest rice (*Oryza sativa*) producer in the country based on planted area (USDA, 2018). Though studies using cereal straw to prevent algal growth have concentrated on barley straw, other cereal grain straws may be effective substitutes for barley straw because similar chemicals are produced during decomposition (Newman, 2004; Park et al., 2006). The large quantities of rice straw in Arkansas make rice straw favorable when attempting to minimize the cost of algal control. Furthermore, cyanobacterial populations in rice paddies were less dense in the second year of cultivation than in the first year when residues from the first year were left in the paddies, lending support to the hypothesis that rice straw is effective at inhibiting algal growth (Rice et al., 1981).

Materials and Methods

Experimental Setup

After presenting the project background, research questions, and anticipated experimental approach to the Ozark Cattlemen’s Association and faculty, five volunteers agreed to participate in the project by granting access to their ponds. Nine farm ponds in Washington County were selected for this experiment (Table 1; Fig. 1). For two properties, three ponds were located on one property and each treatment was randomly assigned to a pond on the property. For the last three remaining ponds located on different properties, each treatment was assigned randomly to a pond (Fig. 1). Ponds treated with barley straw were labeled pond B1, B2, and B3. Ponds treated with rice straw were labeled pond R1, R2, and R3, and the three ponds left untreated as an experimental control were labeled pond C1, C2, and C3. The surface area was calculated for all ponds by measuring the length and width with a tape measure. Ponds with straw were treated at a rate of 25 g/m² with oven-dried straw (Abou El Ella et al., 2007).

The appropriate masses of barley and rice straw were portioned for the respective ponds, cut into pieces approximately 15 cm in length, and placed into plastic 0.5-cm mesh bags. Bags were packed loosely, so water could easily flow through the bag and contact the decomposing straw. When filled with straw, bag volume approximated 90 cm by 55 cm by 40 cm. Pool noodles were tied to the bags with twine to keep the bags afloat in the ponds and promote conditions for aerobic decomposition. Bags were placed on their sides so that the bottom of the bag was at a depth of approximately 20 cm. Each straw bag was anchored to

the pond floor using bricks tied to the ends of string stabilizing placement and evenly spacing bags within ponds. Due to varying pond size, the number of straw bags per pond ranged from one bag in pond R1 to eight bags in pond B2. Brick anchors were attached by a length of string equal to the pond depth at the location of each straw bag. Barley and rice straw bags were placed in ponds on 12 June 2018.

Sampling

Beginning on 12 June 2018, when the rice and barley straw were placed in each respective pond, water samples were collected weekly from each of the 9 ponds for 14 weeks. Ponds were sampled in the order: R1, B1, C1, R2, B2, R3, B3, C3, C2. Composite samples consisted of 5 individual 125-mL samples collected at a depth of 15 cm (625-mL total sample) at regular intervals across a transect dissecting each pond. Individual sample locations corresponded to the following: 1) close to the pond bank, 2) a quarter of the distance across the pond, 3) the center of the pond, 4) three-quarters of the distance across the pond, and 5) at the opposite bank. Samples were collected traversing each pond in an aquatic sampling vessel to prevent water and sediment disturbance. Samples were immediately covered with aluminum foil to prevent further photosynthesis and photodegradation. The final date of sampling was 17 September 2018.

Chlorophyll-a

To measure chlorophyll-a concentrations, 50 mL from each composite pond sample were filtered in the field using a hand pump and GF/F filter (Whatman, 0.7- μ m pore size). Filtrate was saved for further filtration for NO_3^- -N and phosphorus analysis. After returning to the laboratory, each filter was soaked in 7 mL of 90% acetone for 24 hours and stored in a freezer. The extract was analyzed using a Trilogy Laboratory Fluorometer (Turner Designs, San Jose, Calif.). The “Chl-a” module of the fluorometer was calibrated using a stored calibration curve. After samples had been equilibrated to room temperature, extract from each sample (3 mL) was pipetted into a culture tube. Each tube was placed into the fluorometer one at a time. The sample was measured before acidification. After the measurement was complete, 0.1 mL of 0.1 N hydrochloric acid was pipetted into the tube. Following a 90-second reaction period, the sample was measured after acidification. The acidification step converts all chlorophyll-a to pheophytin, a degradation product of chlorophyll-a, for conversion to a pheophytin-corrected chlorophyll-a concentration measured by the fluorometer. Resulting chlorophyll-a ($\mu\text{g/L}$) concentrations were recorded.

On week 14 (17 September 2018), nine 50-mL water samples were collected at a depth of 15 cm in pond B2 to evaluate spatial distribution of chlorophyll-a concentrations. Water samples were collected at distances of 0, 3, and

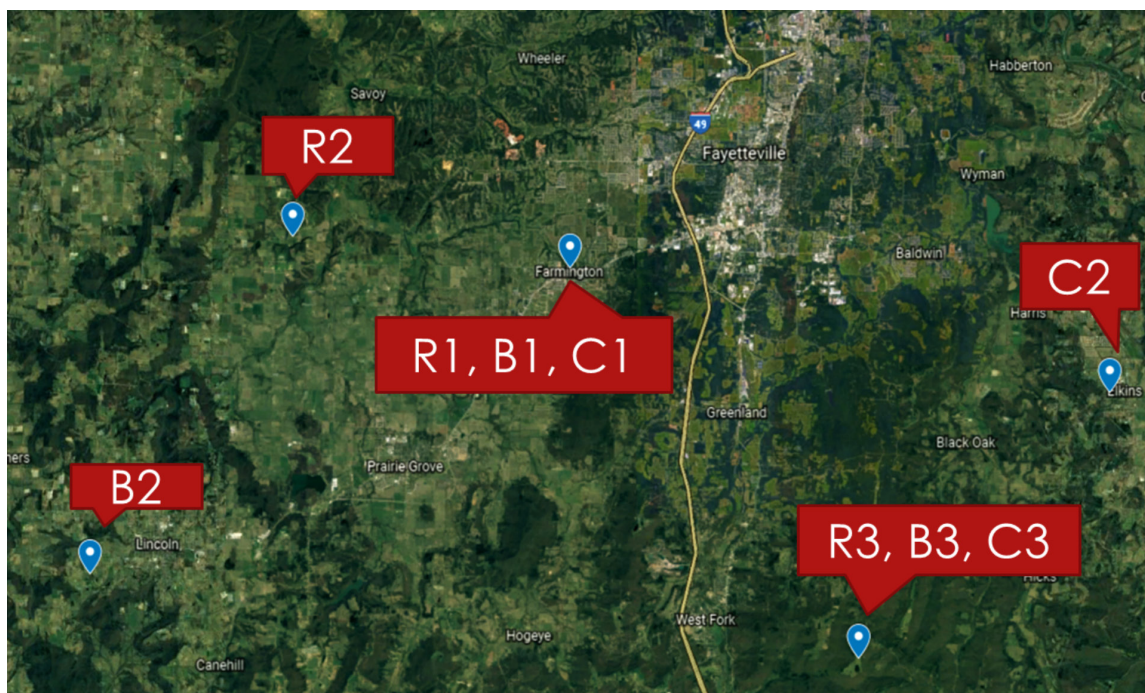


Fig. 1. Map showing the location of each pond studied in Washington County from Google Earth (2019). Ponds R1, R2, and R3 were treated with rice straw. Ponds B1, B2, and B3 were treated with barley straw. Ponds C1, C2, and C3 were left untreated as a control.

6 m from three separate straw bags for a total of nine samples. Each sample was filtered in the field, prepared, and measured for chlorophyll-a concentration as described previously.

Nitrate-Nitrogen (NO₃⁻-N) and Dissolved Phosphorus

Using a 0.45-μm pore size nylon syringe filter (Cole Parmer, Vernon Hills, Ill.), 10 mL of filtrate from the chlorophyll-a procedure were filtered before leaving the field. Two drops of 5-M hydrochloric acid (HCl) were added to each sample after filtration to preserve the sample. Nitrate-N concentrations were measured using cadmium reduction and the modified Griess reaction on a Sans-plus segmented-flow autoanalyzer (Skalar Inc, Buford, Ga.) (Baker et al., 2018). The calibration curve was prepared from 0-, 1-, 2-, 5-, 8-, and 10-mg/L standards. The filtered and acidified water samples that did not produce an instrument response were recorded as a concentration of 0 mg NO₃⁻-N/L.

Filtered and acidified water samples were sent to the Agriculture Diagnostic Laboratory at the University of Arkansas System Division of Agriculture Agricultural Experiment Station to be analyzed for dissolved phosphorus on a Spectro Arcos inductively coupled plasma-optical emission spectrometer (ICP-OES) (SPECTRO Analytical Instruments, Kleve, Germany).

Water Quality Parameters

Dissolved oxygen was measured in-situ at each of the 5 locations along the sampling transect, at a depth of 15 cm, using a Lab Quest 2 (Vernier Software & Technology, Beaverton, Ore.) and Vernier Dissolved Oxygen Probe, and values were averaged for each pond. The pH, temperature, and turbidity were measured on the composite samples in the field using a pH Sensor, Stainless Steel Temperature Probe, and Turbidity Sensor (Vernier Software & Technology, Beaverton, Ore.).

Decomposition

To measure the decomposition of straw in each pond, the initial dry weight of straw was measured before adding to each pond. After the 14 weeks, the straw was removed from the ponds, dried in drying ovens at 55 °C for three weeks, and weighed again. Percent decomposition was calculated by subtracting the final weight from the initial weight, dividing the difference by the initial weight and multiplying by 100.

Precipitation

Precipitation data were obtained from the Town Branch at Armstrong Street weather station in Fayetteville, Arkansas on the United States Geological Survey website (USGS, 2018).

Data Analysis

Chlorophyll-a concentrations were converted to relative percent difference from week 1 concentrations for each pond according to Eq. 1.

$$\frac{x - x_0}{\left(\frac{x + x_0}{2}\right)} * 100 \quad \text{Eq. 1}$$

where x_0 was the chl-a value in week 1 and x was the chl-a value of the current week.

Averages, standard deviations, and standard error of the mean were calculated each week for the average relative percent difference in chlorophyll-a from week 1, NO₃⁻-N, phosphorus, dissolved oxygen, pH, temperature, and turbidity.

Data organization, graph creation, and data analysis were conducted in Excel 2016 (Microsoft Corp., Redmond, Wash.). Repeated measures analysis of variance (ANOVA) tests were performed on each dependent variable to determine statistical significance at $\alpha = 0.05$. Bonferroni post-hoc analysis was conducted on variables with significant P -values. Statistical analyses were used to determine if dependent variables differed across treatments over time. A t -test was used to determine the statistical significance of straw decomposition between rice and barley straw ($\alpha = 0.05$). A single factor ANOVA was used to determine statistical significance among chlorophyll-a concentrations sampled at increasing distances from the straw bag ($\alpha = 0.05$). Linear regression was used to determine if dissolved oxygen and temperature changed through time at a 95% confidence level.

Results and Discussion

During the 14 weeks, $28.5 \pm 19.3\%$ (average \pm standard deviation) of the barley straw placed in ponds decomposed, while $43.7 \pm 13.4\%$ of the rice straw decomposed. Decomposition was not significantly different ($P = 0.26$). Barley straw decomposed to the same extent as rice straw (~40%) in two of the ponds; however, pond B3 resulted in only a 6.7% decrease in barley straw. The dissolved oxygen was consistently low, usually between 3 and 4 mg/L in pond B3. The range for dissolved oxygen concentration in ponds with rice straw and the control was 4.1 mg/L to 6.5 mg/L and 3.9 mg/L to 6.7 mg/L, respectively (Fig. 2). Pond B3 also had a layer of accumulated leaf litter on the bottom of the pond; therefore, aerobic microbial activity in B3, and thus aerobic decomposition of barley straw and production of any allelopathic compounds, may have been more constrained by abiotic conditions in the pond compared to other ponds.

Dissolved oxygen concentration differed among the rice straw and barley straw treatments and the control ($P < 0.001$). Time affected dissolved oxygen ($P = 0.02$). Dis-

solved oxygen concentration in the barley straw treatment (4.26 ± 0.65 mg/L average \pm standard deviation) was different than dissolved oxygen in both the rice straw treatment (5.44 ± 0.73 mg/L average \pm standard deviation) and the control (5.47 ± 0.76 mg/L average \pm standard deviation). Dissolved oxygen concentration in week 1 (12 June 2018) differed from weeks 4, 5, 7, and 8. Dissolved oxygen concentration in week 2 (18 June 2018) differed from weeks 4, 5, and 8, and week 4 differed from weeks 11, 12, and 13.

Lower dissolved oxygen concentration in the barley straw treatment could decrease the decomposition rate of barley straw in ponds. Although the average dissolved oxygen concentrations varied during the study, the average concentrations remained above 3.6 mg/L, which is sufficient for aerobic decomposition to occur (Cech, 2010). The differences in dissolved oxygen over time could be caused by the changes in water temperatures as the summer progressed. There could have been temporal or spatial locations in at least some of the ponds in which low oxygen concentrations were limiting to the efficacy of straw decomposition to control algal growth.

Initial chlorophyll-a concentrations ranged from 18.8 μ g/L in pond B1 to 457 μ g/L in pond R2. Within the rice treatment, the initial range of concentrations was 436 μ g/L. Ponds treated with barley had an initial range of 255 μ g/L, and control ponds had an initial concentration range

of 108 μ g/L across the three ponds. The relative percent differences in chlorophyll-a concentrations from week 1 fluctuated through time in all treatments (Fig. 3).

In week 14, the final sample date, both treatments and the control had negative relative percent differences from the week 1 concentration, meaning that there was less algal biomass in week 14 than week 1 in both treatments and the control. In the rice treatment, the relative percent difference ranged from -90.1% to 69.8%. In the barley straw treatment, the relative percent differences ranged from -119% to 23.7%. In the control group, the relative percent differences ranged from -127% to 80.2%. The relative percent difference in chlorophyll-a from week 1 of sample collection did not differ statistically between the straw treatments or between the treatments and the control ($P = 0.85$, Table 2). During the 14-week study, there were seven weeks when relative percent differences in all treatments were negative, meaning average chl-a concentrations were less than week 1, two weeks when the rice straw group was positive, meaning average chl-a showed growth compared to week 1, three weeks when the barley straw group was positive, and four weeks when the control group was positive. Thus, there was no indication of consistent control of algal biomass in either straw treatment, nor was there any consistent trend with algal biomass growth throughout the 14-week experiment ($P = 0.69$, Table 2).

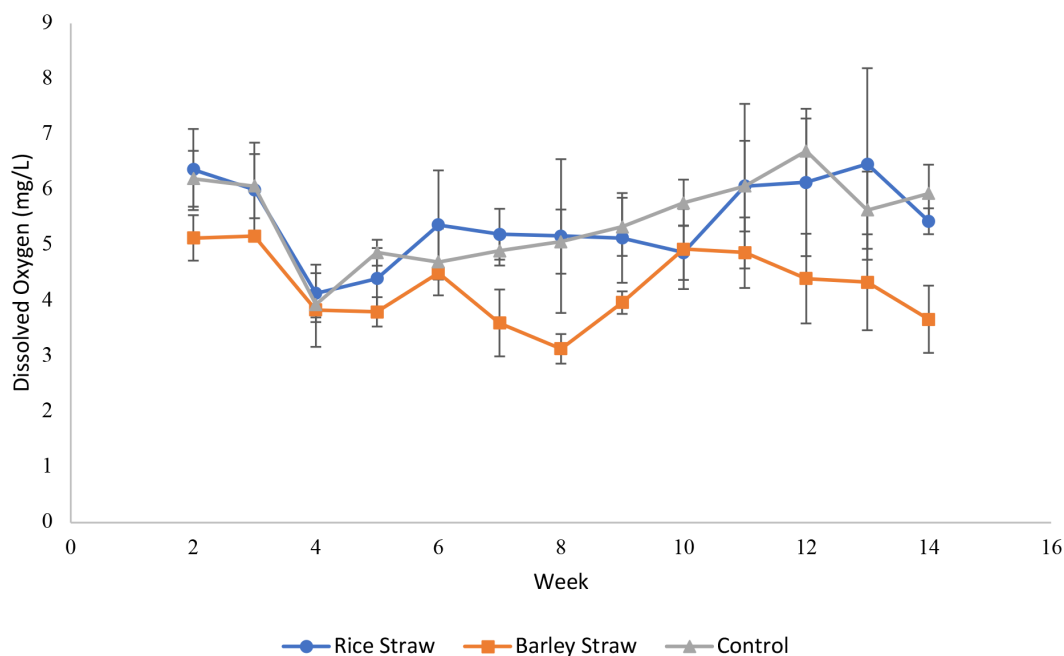


Fig. 2. Dissolved oxygen concentrations (mg/L) in ponds treated with rice straw, barley straw, and no treatment (control) during the fourteen-week study from 12 June 2018 to 17 September 2018. Samples for each treatment were averaged ($n = 3$). Error bars are standard error of the mean.

The variability in chlorophyll-a among ponds within the same treatment could have been caused by environmental factors, such as pond sediment composition, the type and proximity of livestock to the ponds (Table 1), or the flow rate of water within the ponds, factors that were not quantified in this study. For example, pond R2 was spring-fed and feeds into an ephemeral stream. Relative percent difference in chlorophyll-a concentration was negative in all weeks after week 2, indicating algal inhibition throughout the study in pond R2 containing rice straw despite ducks, geese, and cattle having direct access to the pond (Table 1). The movement of water flowing across the pond may have circulated inhibitory chemicals from the decomposing straw throughout the pond. Abou El Ella et al. (2007) controlled algal growth with cereal straw in the Suez Canal, where wave and wind action caused consistent mixing of the water. However, other studies have shown that cereal straw is effective in lentic pond systems as well (Islami and Filizadeh, 2011). Therefore, the efficacy of cereal straw to inhibit algal growth was not expected to be dependent on circulation of water; however, diffusion of inhibitory compounds within farm ponds may be a consideration that requires further investigation.

There was no difference in chlorophyll-a concentrations with distance from the straw bags as measured at 0, 3, and 6 m in pond B2 on week 14 (17 September 2018) (P

$= 0.49$). Average chlorophyll-a concentrations were 41.98 ± 10.76 , 41.86 ± 15.63 , 52.78 ± 9.30 $\mu\text{g/L}$ (average \pm standard deviation) at 0, 3, and 6 m distance from straw bags, respectively. Lack of difference among chlorophyll-a concentrations at different distances from straw bags indicates that diffusion of chemicals dissipating away from the decomposing straw source was not the limiting factor to the efficacy of straw as an algal growth inhibitor in the pond environment.

Water temperature was measured as a factor that could influence decomposition, the algal community, and dissolved oxygen concentrations. Both the treatment ($P = 0.01$, Table 2) and time ($P < 0.001$) affected water temperature (Fig. 4). There was no difference among temperatures in the rice and barley treatments; however, both the rice and barley treatments differed from the control. Water temperature in week 2 (18 June 2018) differed significantly from water temperature in week 13. Water temperature in week 3 (7 July 2018) differed from weeks 7, 10, and 13, week 4 (16 July 2018) differed from weeks 7 and 13, and week 8 (6 August 2018) differed from water temperature in week 13, respectively. Water temperature in week 11 (27 August 2018) differed from weeks 1, 5, 6, 7, 9, 10, and 13; week 12 (5 September 2018) differed from weeks 7, 9, 10, and 13; week 14 (17 September 2018) differed from water temperature in week 7, respectively.

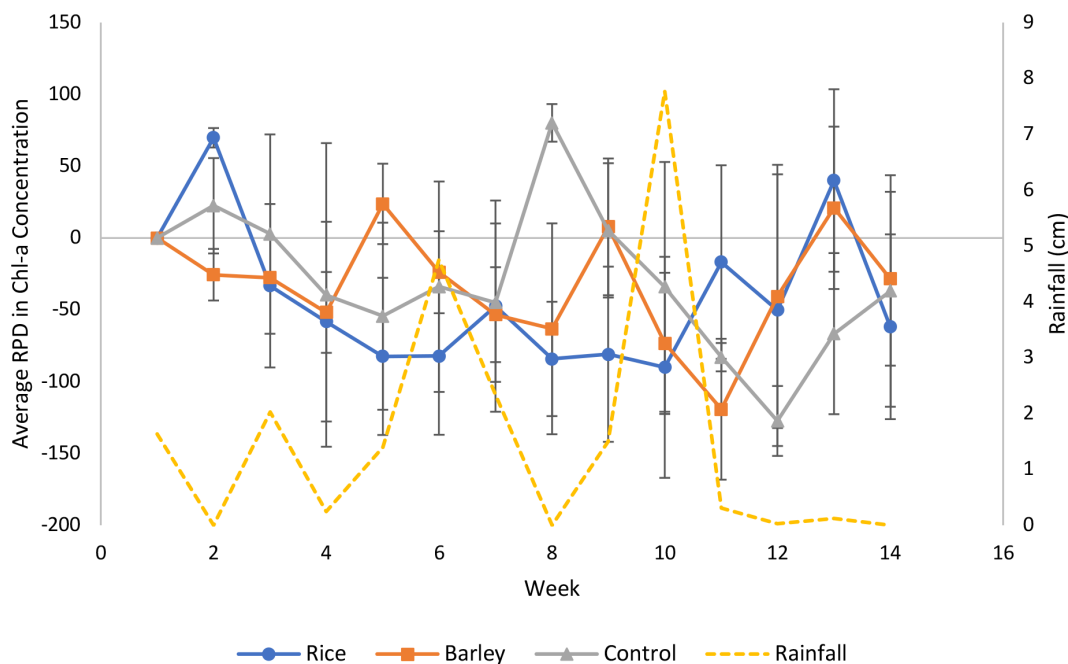


Fig. 3. Relative percent difference (RPD) from week 1 of chlorophyll-a concentrations ($\mu\text{g/L}$) in ponds treated with rice straw, barley straw, and no treatment (control) with weekly rainfall data in cm (USGS, 2018) during the 14-week study (12 June 2018 to 17 September 2018). Samples for each treatment were averaged ($n = 3$). Error bars depict standard error of the mean.

Table 1. City name, Global Positioning System (GPS) coordinates, surface area, mass of straw added, and the surrounding land use of each pond in the study. Ponds R1, R2, and R3 were treated with rice straw. Ponds B1, B2, and B3 were treated with barley straw. Ponds C1, C2, and C3 were left untreated as a control.

Pond ID	City	GPS Coordinates for each Pond	Surface Area	Straw Added	Surrounding Land Use
			m ²	kg	
R1	Farmington, Ark.	36°01'49.6"N 94°14'16.9"W	230.8	5.770	Horse and donkey pasture with access to pond Occasional cattle
R2	Farmington, Ark.	36°03'12.2"N 94°21'45.2"W	670.7	16.768	Cattle pasture with access to pond Domestic ducks and geese nesting on pond bank
R3	West Fork, Ark.	35°54'42.7"N 94°07'22.5"W	1514.9	37.870	Cattle pasture with access to pond Occasional wild ducks in pond
B1	Farmington, Ark.	36°01'53.8"N 94°14'17.5"W	414.8	10.369	Horse and donkey pasture with access to pond Occasional cattle
B2	Lincoln, Ark.	35°56'19.1"N 94°27'08.1"W	286.5	70.911	Cattle pasture with access to pond
B3	West Fork, Ark.	35°54'45.0"N 94°07'32.2"W	1631.8	40.795	Cattle pasture with access to pond
C1	Farmington, Ark.	36°01'55.4"N 94°14'11.4"W	1436.6	0	Horse and donkey pasture with access to pond
C2	Elkins, Ark.	36°00'02.3"N 94°00'53.3"W	2251.4	0	Cattle pasture with access to pond Ranging chickens
C3	West Fork, Ark.	35°54'22.6"N 94°07'35.8"W	2302.7	0	Cattle pasture with access to pond

Table 2. Analysis of variance summary of the effects of straw treatment and time on properties measured in pond water.

Dependent variable	P-value		
	Straw treatment	Time	Straw treatment by time
Chlorophyll-a	0.845	0.694	0.909
Dissolved oxygen	<0.001**	0.019*	0.997
Temperature	0.009*	<0.001**	0.998
NO ₃ -N	<0.001**	0.976	0.998
Phosphorus	0.024*	0.274	0.971
pH	0.090	0.202	0.890
Turbidity	0.081	0.466	0.660

* $P < 0.05$; ** $P < 0.001$.

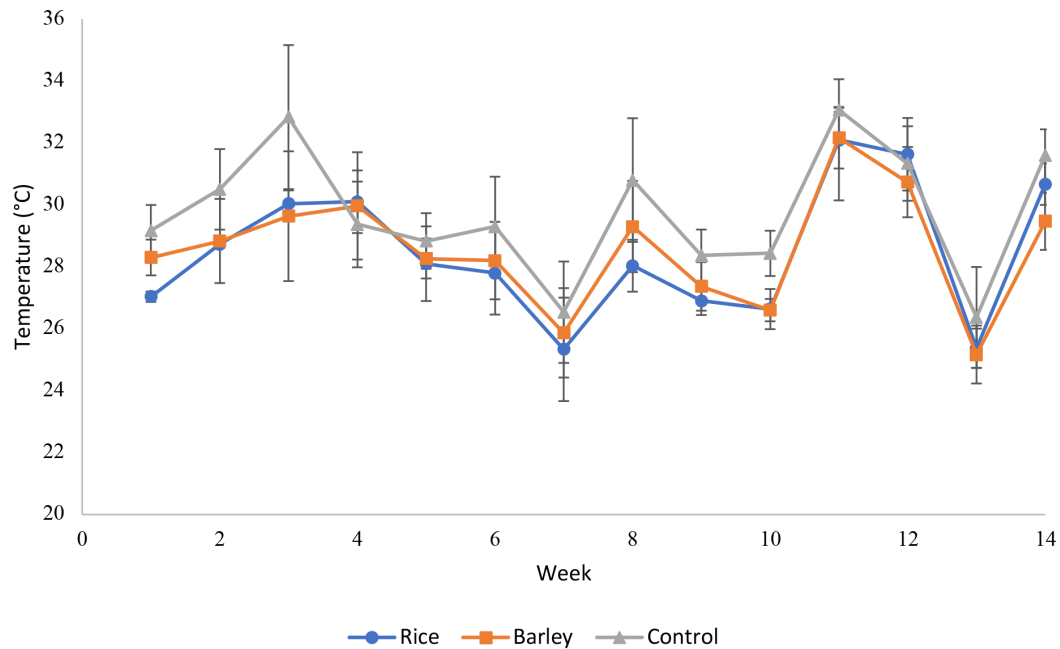


Fig. 4. Temperature (°C) in ponds treated with rice straw, barley straw, and no treatment (control) during the 14-week study from 12 June 2018 to 17 September 2018. Samples for each treatment were averaged ($n = 3$). Error bars are standard error of the mean.

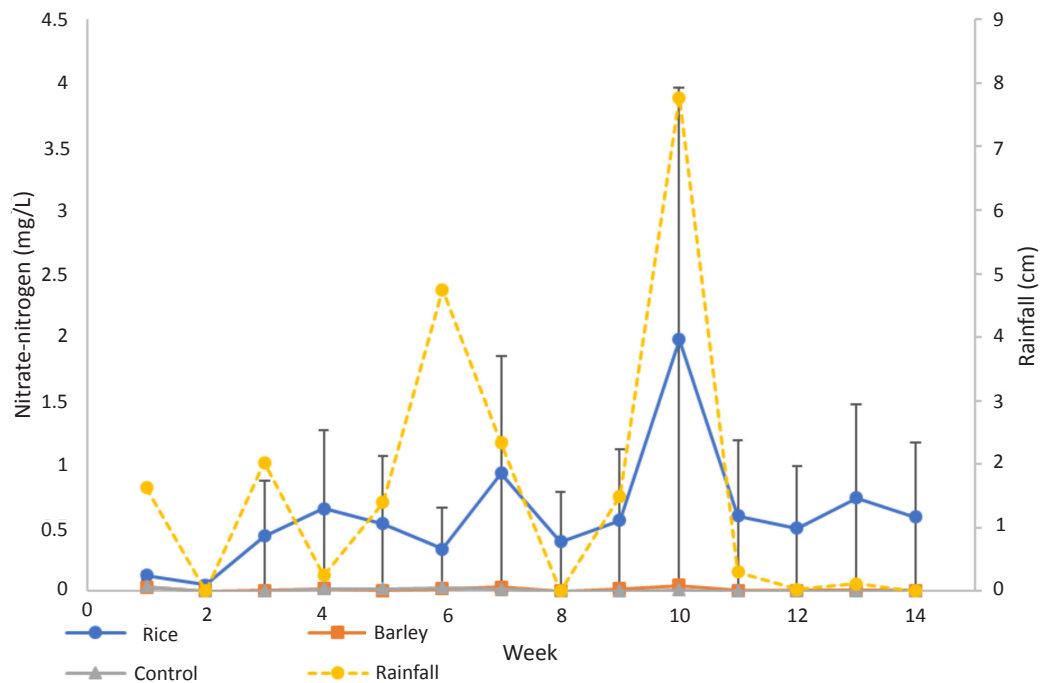


Fig. 5. Nitrate-nitrogen concentrations (mg/L) in ponds treated with rice straw, barley straw, and the control (no treatment) with weekly rainfall data in cm (USGS, 2018) during the 14-week study from 12 June 2018 to 17 September 2018. Samples for each treatment were averaged ($n = 3$). Error bars are standard error of the mean.

The greater temperature in the control ponds could have been due to surrounding land management or an artifact of the sampling procedure. Control ponds lacked tree cover on the banks. More direct sunlight would increase water temperature. The last two ponds sampled each day were both in the control group, so the ponds had more time to warm throughout the day. Randomizing the order in which the ponds were sampled would have controlled for the effect of time of day on water temperatures; however, sampling order was chosen using the most efficient route between ponds to assure all samples could be collected on the same day.

Straw treatment significantly affected NO_3^- -N ($P = 0.0003$) and dissolved phosphorus ($P = 0.02$) concentrations, but concentrations did not differ across sampling times ($P = 0.98$ for NO_3^- -N and $P = 0.27$ dissolved P measurements across time, respectively, Table 2). Ponds containing rice straw had greater concentrations of NO_3^- -N than ponds containing barley straw or the control (Fig. 5). Average NO_3^- -N concentrations in ponds containing barley straw and the control were 0.013 and 0.009 mg/L, respectively. Average NO_3^- -N concentration in rice straw-treated ponds was 0.599 mg/L. In the rice straw treatment, average dissolved phosphorus concentration was 0.097 mg/L and did not differ from the control which averaged

0.031 mg/L (Fig. 6). In the barley straw treatment, average phosphorus concentration was 0.123 mg/L, which was greater than the control.

Differences in NO_3^- -N concentrations among treatments could have been due to the type and proximity of livestock to ponds. Cattle had access to all ponds (Table 1). Pond C2 had chickens roaming near the pond; although, the chicken house was downslope from the pond. Horses and donkeys were in fields adjacent to ponds R1, C1, and B1, but were never observed in the water on sampling dates. Pond R2 had domestic ducks and geese that nested on the bank of the pond, and pond R3 occasionally had wild ducks feeding in the pond. The waterfowl in ponds R2 and R3 might explain the greater concentration of NO_3^- -N in the rice straw treatment. Low NO_3^- -N levels in the control and barley straw ponds could indicate that the conditions necessary for algal growth were not present. The ideal nitrate-to-phosphate ratio by mass for algal growth is approximately 10:1, and concentrations of individual nutrient requirements vary among algal species (Downing and McCauley, 1992). During no week in either treatment or the control was NO_3^- -N concentration great enough to achieve the ideal 10:1 nitrate-to-phosphate ratio for algal growth. Nutrient availability may have contributed to the lack of statistical differences in chlorophyll-a concentrations.

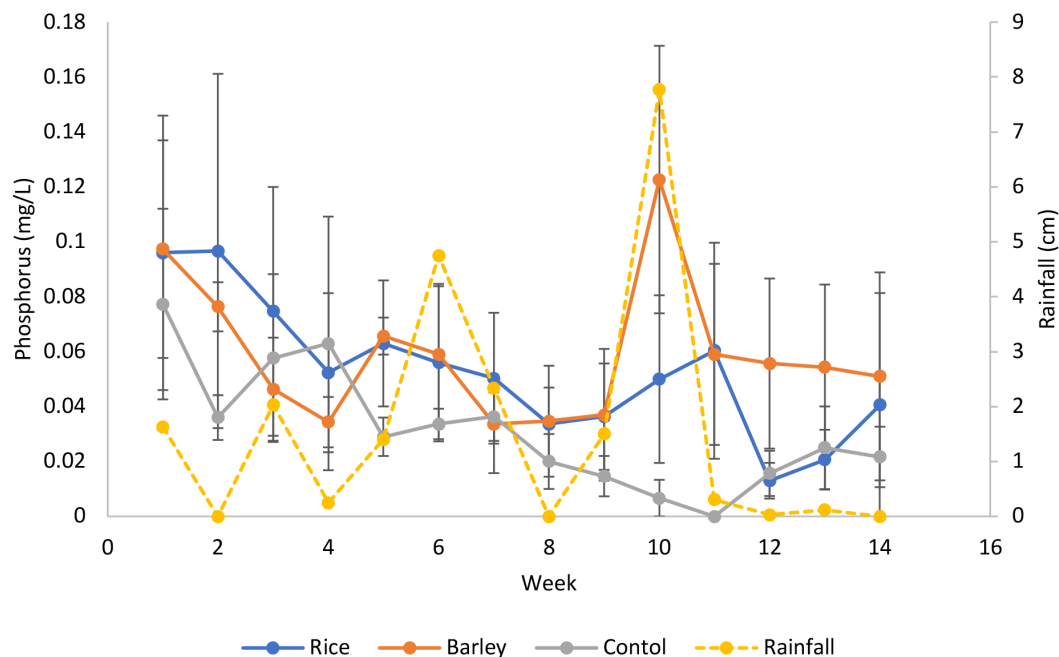


Fig. 6. Dissolved phosphorus concentrations (mg/L) in ponds treated with rice straw, barley straw, and the control (no treatment) with weekly rainfall data in cm (USGS, 2018) during the 14-week study from 12 June 2018 to 17 September 2018. Samples for each treatment were averaged ($n = 3$). Error bars are standard error of the mean.

Conclusions

Due to the lack of differences in chlorophyll-a among the treatments and control, neither rice straw nor barley straw was effective at inhibiting algal growth in the farm ponds studied. Since there was no difference between the rice straw and barley straw treatments, it is unclear if rice straw is as effective as barley straw at inhibiting algal growth. Further research is needed to determine the efficacy of cereal grain straw as a reliable method of algal biomass control.

Acknowledgements

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Increasing low-income residents' access to fresh produce through a local mobile pantry

Meet the Student-Author



Laura Wasson

Research at a Glance

- This study aims to determine whether mobile pantries are an effective method to reduce food insecurity and increase fruit and vegetable consumption in low-income communities.
- A sample of low-income residents in Washington County, Arkansas was surveyed after receiving a donation from a local mobile produce pantry revealing an improvement in diet after receiving approximately 1–2 cups of fruits and/or vegetables.
- Mobile pantries focused on distributing fresh produce are an effective way to increase low-income community's fruit and vegetable consumption and meet the USDA Food Patterns recommendations.

After grasping nutrition's crucial role in overall health and well-being and learning of the disparity in nutrition knowledge and access that spans the globe and even my home state of Arkansas, I knew immediately that I wanted to use my education to minimize this gap.

I wanted to directly impact the local food-insecure community. Networking through the Business College's Social Innovation Hub, I met Margaret Thomas and Alyssa Snyder, cofounders of Seeds that Feed. They share my passion for food security and use their mobile food pantry to combat food insecurity in Northwest Arkansas. My research with them has allowed them to improve their service to NWA and further support the role of mobile pantries in reducing food insecurity. I shared my research findings at the Academy of Nutrition and Dietetics' annual conventions (state and national levels) providing a model for others in the field to adopt.

I had the opportunity to travel to Chile where I aided in pediatric nutrition care and to Mozambique where I assisted in poverty and malnutrition relief efforts. I will complete a Dietetic Internship at the Vanderbilt University Medical Center after which I plan to become a Registered Dietitian.

I would like to thank Mechelle Bailey for her investment in my personal and professional development. I also thank Dr. Nalley for assisting me with statistics and data analysis and providing the opportunity to study abroad. I thank Dr. Hill for advising me in my academic pursuits and shaping me to be a professional in the field.



Laura receiving the Outstanding Dietetic Student of the Year (undergraduate) award with her mentor Mechelle Bailey at the Arkansas Academy of Nutrition and Dietetics annual convention.

Increasing low-income residents' access to fresh produce through a local mobile pantry

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Mechelle L. Bailey[§], and Laura L. Hill[‡]*

Abstract

Seeds that Feed (STF) is a mobile food pantry located in Fayetteville, Arkansas. Seeds that Feed receives produce from local farmers to distribute to residents in low-income housing sites throughout Northwest Arkansas. According to Feeding America, food insecurity affected 14.3% of Washington County, Arkansas' population in 2016. The purpose of this study was to determine if STF's model is an effective way to increase individuals' access to fresh fruits and vegetables and increase their potential to meet the United States Department of Agriculture's Food Patterns (USDA-FP) for fruit and vegetable consumption. Twenty-three participants from three sites completed the study. A survey was used to collect basic demographics and dietary patterns. A record was taken of what foods each participant received on the survey day including plans for preparation and to whom it would be served. The results indicated that the likelihood to meet the USDA-FP for overall fruit and vegetable intake increased significantly after receiving approximately one cup of fruit and 1.5 cups of vegetables from STF. A positive correlation was found between the number of times participants received produce from STF and participant's total fruit intake and total intake of the "red/orange" and "other" vegetable subgroups. Therefore, STF's model appears to be an effective method to increase access to fresh produce. Future research could utilize STF's model to assess the potential for other supplemental nutrition programs to help low-income residents meet the USDA-FP and reduce food insecurity via mobile pantries.

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Introduction

According to the United States' largest domestic hunger-relief organization, Feeding America, 12.9% of Americans were food insecure in 2016 (Feeding America, 2018). Nunnery et al. (2018) defined food insecurity as "the condition of inconsistent or uncertain availability of safe and nutritious adequate food". Food insecurity stems from a multitude of factors including income, socio-economic status, race, access, and transportation. Income influences food purchases and thus, is an indicator for predicting dietary patterns (Tsang et al., 2011; Strome et al., 2016; Singleton et al., 2017). Low-income status is commonly associated with a diet greater in energy-dense, highly processed foods with little nutritive value (Dutko, et al., 2012; Strome, et al., 2016; Nunnery et al., 2018). Multiple studies conclude that income is often the limiting factor preventing low-income status populations from purchasing enough fruits and vegetables to meet the Dietary Guidelines for Americans (DGA) set by the USDA (Zepeda et al., 2014; Nunnery et al., 2018, USDHHS and USDA, 2015).

The USDA divides vegetables into five subgroups based on their varying nutrient profiles; these subgroups are dark leafy greens, red and orange vegetables, other vegetables, starchy vegetables, and beans and legumes. Only about one-fourth of the population meets the USDA recommendations putting the nation at a deficit for both fruit and vegetable consumption. Regular consumption of fruits and vegetables that meet the recommendation set by the USDA largely influences the progression and prevention of chronic illness; therefore, intervention programs that focus on providing consistent access to affordable, nutrient-dense produce is of utmost concern for the health of our nation (USDA, 2017; Strome et al., 2016; Nunnery et al., 2018).

Improving access to fruits and vegetables is a primary factor for increasing consumption. Transportation also is often recognized as one of the largest barriers to accessing fresh produce (Dunn et al., 2011, Tsang et al., 2011). An individual may live within a few miles of a large grocery store, but not having a car limits the quantity one can purchase in a single trip (Strome et al., 2016). Mobile pantries are proven to minimize the gap between residents and available produce. Multiple studies show that the use of a mobile pantry to distribute discounted produce to food insecure, low-income neighborhoods can increase residents' consumption of fresh fruits and vegetables (Zepeda et al., 2014; Hosler and Kammer, 2015).

In this study, researchers observed the work of a mobile pantry in Northwest Arkansas, Seeds that Feed (STF), in order to determine its effectiveness in increasing fruit and vegetable consumption among low-income residents in Northwest Arkansas. In Washington County Arkansas (the location of the current study), 14.3% of the popula-

tion was considered to be food insecure in 2016 (Feeding America, 2018). In 2016, three-quarters of residents in Washington County were eligible for food assistance programs based on income (Feeding America, 2018). Seeds That Feed identified multiple low-income housing sites across Northwest Arkansas to deliver fresh produce to its food-insecure residents. This study aimed to determine if STF's mobile pantry was effective in increasing access to fresh produce for food-insecure residents in Northwest Arkansas. Related trends and barriers were also assessed.

Materials and Methods

Upon receiving approval from the University of Arkansas' Institutional Review Board (IRB), a survey was created through the software Qualtrics (Qualtrics LLC, Provo, Utah) and distributed to participants at three of STF's mobile pantry donation sites on three separate days. These sites included a senior citizen assisted-living apartment complex, a low-income family housing complex, and the Springdale, Arkansas Women, Infants and Children (WIC) clinic. Seeds That Feed receives its produce from farmers and some food stores in Northwest Arkansas who have agreed to donate a portion of their produce and other food items to the mobile pantry. Seeds that Feed then delivers these food items to partnering low-income housing complexes in Northwest Arkansas at no cost to its residents. The researchers accompanied STF staff to distribution sites where participants were allowed to take as little or as much produce as they wanted. Participants were considered eligible for the study after collecting produce from STF and were recruited on a volunteer basis resulting in a convenience sample of 23 total participants. Researchers approached participants after they received their produce collection to explain the survey, request their participation, and receive their verbal consent before distributing the survey.

Two of the observed sites had consistently received donations from STF preceding this study. Because most of the participants from the first two locations, the senior citizen assisted-living apartment complex and the low-income family housing, were returning customers, these participants were informed in advance that STF would be conducting a survey at the visit and that their participation was a way to "return the favor" to STF and help STF serve them better. However, it was made clear to all participants there were no penalties for not participating. The WIC clinic and its members had no prior relationship with STF leading up to this study. Participation was completely voluntary and thus, participants were able to exit the survey at any time without any consequences.

Data collected included a range of basic demographics information. Data collected also included a food frequency record, and how frequently returning customers received

produce from STF. Participants were also asked to indicate where they shopped for food, what barriers they faced when trying to access food, and what, if any, supplemental nutrition program(s) they used. Lastly, participants were asked to specify what produce they received on that day from STF.

The survey was used to determine each participant's average dietary intake in relation to the USDA-FP for their respective age and gender. These data revealed participants' average dietary intake of fruits, vegetables, and vegetable subgroups as well as the potential increase in their dietary intake of fruits, vegetables, and vegetable subgroups after receiving the produce from STF.

Each participant's answers to the food frequency record were recorded and standardized as a one-cup serving size for fruit and vegetable subgroups. Servings were standardized to one cup in order to correlate with the measurements utilized by the USDA-FP for fruit and vegetable servings. Participants' answers to the food frequency record portion of the survey were compared to the USDA-FP to determine if their average weekly fruit and vegetable intake met or fell below the USDA-FP before receiving a donation from STF according to their age and gender (USDA, 2017). When determining whether individual participants met the USDA-FP, gender and age were taken into account. Participants also recorded the foods they received, how they intended to prepare them, and the number of children and adults to whom they would be served. These responses were then analyzed and factored into participants' answers from the food frequency record. These data were used to determine if each participant's potential to meet the USDA-FP increased after receiving a donation from STF and to what degree. These outcomes and correlations were determined using Microsoft Excel.

Results and Discussion

The goals of this study were to collect data related to participants' diet and barriers in accessing fresh produce so that STF and other mobile pantries may expand upon their services to reduce food insecurity. Specifically, the study aimed to determine if there was a correlation between demographics and the amount of fresh produce consumed by STF participants. Further, the study wanted to determine if STF's mobile pantry was effective in increasing access to fresh produce for food-insecure residents in Northwest Arkansas. Lastly, the study set out to determine if the number of times a participant has received donations from STF positively correlated with their consumption of fresh produce. The results from this study can be used by STF, other mobile food pantries, policymakers and advocates to better understand the impacts that mobile food pantries can have on increasing fruit and vegetable consumption.

According to participants' self-reported answers to the food frequency record, none of the USDA-FP of the fruit and vegetable groups represented in the study were met by all of the participants (Table 1). In other words, at least one—and in most cases more than one—participant fell short of the USDA-FP for every fruit and vegetable group represented in the study. Twenty-one of the 23 (91%) participants did not meet the USDA-FP for one or more of the fruit and vegetable groups represented in this study. In fact, on average, participants did not meet the USDA-FP for three of the seven fruit and vegetable groups, and at the low-income family housing location, the average was four out of seven fruit and vegetable groups unmet.

Participants received approximately 1 cup of fruit total and 1.5 cups of vegetables total from STF. From these

Table 1. Percentage of participants who met the United States Department of Agriculture Food Patterns (USDA-FP) for overall fruit, overall vegetable, and vegetable subgroup intake before receiving a donation from Seeds That Feed (STF) compared with the percentage of participants who had the potential to meet the USDA-FP after receiving a donation from STF.

Timing & change in consumption	Vegetable subgroups						
	Total vegetable	Total fruit	Leafy green vegetables	Other vegetables	Red/orange vegetables	Starchy vegetables	Beans and legumes
	Percent (%) of participants meeting USDA-FP consumption goal						
Before	49	57	91	61	22	30	70
After	57	61	91	74	22	-	-
Increase	8	4	No change	13	No change	NA ^a	NA ^a

^a NA indicates that this vegetable group was not donated by STF during the study.

amounts alone, the participants' potential to meet the USDA-FP for total fruit, total vegetable, leafy green vegetables, other vegetables, red/orange vegetables increased by 10%, 9%, 66%, 38%, and 21%, respectively (Table 2). Vegetables from the subgroups starchy vegetables and beans and legumes were not donated during this study; therefore, no data could be gathered regarding either of those vegetable subgroups. This means that after receiving approximately one cup of fruit and/or approximately 1.5 cups of vegetables, participants' potential to meet the USDA-FP increased on average by 9% for total fruit and 10% for total vegetables. Likewise, for each participant who received approximately one cup of vegetables from the vegetable subgroups leafy greens, other vegetables, and red/orange vegetables experienced a 66%, 38%, and 21% increase, respectively, in their potential to meet the USDA-FP. This information was gathered by comparing individuals' responses to the food frequency record with each food item they received and the amount that they indicated they would eat themselves.

Before receiving a donation from STF during the study period, the percentage of participants who met the USDA-FP for total vegetable, total fruit, leafy green vegetables, other vegetables, red-orange vegetables, starchy vegetables, and beans and legumes was calculated at 49%, 57%, 91%,

61%, 22%, 30%, and 70%, respectively (Table 1). After receiving a donation, the percentage of participants who had the potential to meet the USDA-FP for total vegetables, total fruit, and other vegetables increased by 8%, 4%, and 13%, respectively. Consequently, 57%, 61%, and 74% of participants had the potential to meet the USDA-FP for total vegetable, total fruit, and other vegetables, respectively after receiving a donation from STF (Table 1). There was no percentage change for leafy green vegetables or red/orange vegetables, as donations were not large enough to lift participants to meeting nutrition targets set by the USDA. Nonetheless, the potential to meet the targets did increase as shown in Table 2.

Nine participants (39% of total) received a donation(s) from STF prior to the study. Two participants had received over 24 donations, three participants had received 6 to 8 donations, and four participants had received 1 to 4 donations (Table 3). The categories of total fruit and other vegetables appeared positively correlated with the number of times participants had received a donation from STF. Those who received more donations from STF were more likely to have met the USDA-FP for total fruit and other vegetable intake (Table 3). Due to the small number of participants represented in this study, more research is required to determine the validity of this conclusion.

Table 2. Average percentage increase in participants' potential to meet the United States Department of Agriculture Food Patterns for each fruit and vegetable group after receiving a donation from Seeds That Feed.

Percentage	Vegetable subgroups						
	Total vegetable	Total fruit	Leafy green vegetables	Other vegetables	Red/orange vegetables	Starchy vegetables	Beans and legumes
Average increase in potential to meet the USDA-FP	10%	9%	66%	38%	21%	NA ^a	NA ^a

^a NA indicates that this vegetable group was not donated by Seeds That Feed during the study.

Table 3. Relationship between the number of times participants received a donation from Seed That Feed (STF) and if they met the United States Department of Agriculture Food Patterns (USDA-FP).

# of times participants received donations from STF	Vegetable subgroups							
	Number of participants	Total vegetables	Total fruit	Leafy green vegetables	Other vegetables	Red/ orange vegetables	Starchy vegetables	Beans and legumes
-----Percent (%) of participants meeting USDA-FP consumption goal-----								
>24	2	50	50	100	100	50	0	0
6-8	3	0	33	100	67	0	67	33
1-4	4	50	25	100	50	25	0	50

Conclusions

This study found that mobile food pantries, even small ones like STF, can have substantial impacts on fruit and vegetable intake among the low-income community. Supplemental nutrition programs that prioritize distributing fresh produce to food-insecure families and individuals via mobile pantry have the potential to increase the low-income population's ability to obtain the USDA-FP recommendations for several fruit and vegetable categories. Even in seemingly affluent areas like Washington County, Arkansas, food insecurity is abundant. This study showed that mobile food pantries are part of a larger solution to help the most vulnerable increase food security while simultaneously providing healthy foods to increase holistic well-being. While mobile food pantries like STF are not the solution to hunger and malnourishment alone, this study shows that they can play an important role in assisting in lowering food insecurity and ensuring that underserved communities can achieve a nutritious diet by making produce more affordable and accessible.

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Determination of optimum harvest date for winter malting quality barley in Northwest Arkansas

Meet the Student-Author



Paul Wolf

I am from White Hall, Arkansas and was homeschooled which allowed me to earn a technical certificate in Metal Inert Gas welding before coming to the University of Arkansas in the fall of 2015. After my first semester as a Crop Science major, I added Animal Science as a second major, with the goal of one day owning a self-sustaining farm. My interest in small grains was sparked after helping Dr. Mason and the wheat breeding program with harvest in 2017. And the next year I was not only helping with harvest but harvesting my own research plots. While at the university, I had the opportunity to study abroad in India to learn about their views on GMOs and their agriculture research system. I also served as an officer in the Crop, Soil, and Environmental Sciences Club, before I graduated cum laude in May 2019 with majors in Crop Science and Animal Science and a minor in Agricultural Business.

I am thankful for the help of Dr. Mason and the University of Arkansas Wheat Breeding Program for their help in guiding me through this project.

After graduation, I am planning to take a year off before pursuing my JD and MBA, with the goal of working as an administrative law judge on the many policy issues that the agriculture sector will face.

Research at a Glance

- Barley is a grain that can be grown across much of the world, but its growth is not tracked in Arkansas.
- The preference of barley for malting makes it a potentially profitable grain for Arkansas.
- Barley is able to meet the germination requirements for malt quality when grown in Arkansas.



Paul collecting barley heading date data in May 2017 at the University of Arkansas System Division of Agriculture's Agricultural Experiment Station.

Determination of optimum harvest date for winter malting quality barley in Northwest Arkansas

Paul D. Wolf^{}, David Moon[†], and Richard Esten Mason[§]*

Abstract

Due to the strict quality requirements, only 10% of worldwide barley is used for malting. As such, malting quality barley comes with a price increase of up to 50% or greater. With the craft brewery industry growing in Northwest Arkansas there is a growing demand for locally sourced malt quality barley. However, data are lacking regarding production practices for barley in Arkansas. The optimal harvest date for malting quality barley is at physiological maturity. This is because many of the malting traits (such as germination energy) decline as the harvest is delayed, which makes it difficult to meet the criteria for malting quality if the barley is left in the field. The purpose of this study was to evaluate the effects of harvest date on the malting quality of barley grown in Northwest Arkansas, specifically, the effect of harvest date on barley seed quality characteristics that impact malting and the interaction of harvest date and cultivar. Harvest date, cultivar, and in many cases the interaction of harvest date and cultivar were significant for grain yield, test weight, water sensitivity, germination energy, and germination capacity. There was no significant variation between cultivars for protein content. In general, all malting quality traits decreased with delayed harvest and the decrease at 21 days after physiological maturity was statistically significant. Of the cultivars tested, Thoroughbred was closest to meeting the criteria for malting quality, having the greatest grain yield, while maintaining germination energy and capacity into a later harvest date.

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[§] Richard Esten Mason, the faculty mentor, is a professor in the Department of Crop, Soil, and Environmental Sciences.

Introduction

Barley is number four in terms of area cultivated in cereal grains in the world at 49.24 million hectares (USDA-FAS, 2019). The major uses of the barley grown is for malting and as a feed source (Jacobs, 2016). Due to the strict quality requirements, approximately 10% of world-wide barley is used for malting, though malting quality barley comes with a price increase of up to 50% or greater. In the United States, 25% of the barley grown is used for malting (Davison et al., 2007). In 2017, 1,004,025 ha of winter and spring barley were planted in the United States, and 790,756 ha were harvested (USDA-FSA, 2018). No barley production for Arkansas was reported to the Farm Service Agency for 2017 (USDA-FSA, 2018).

With the craft brewery industry growing in Arkansas, particularly in the Northwest area of the state where over half of the state's microbreweries reside, there is a growing demand for locally sourced malt quality barley (Brewers Association, 2018). However, data are lacking regarding production practices for barley in Arkansas. The University of Arkansas System Division of Agriculture's Agricultural Extension Service offers handbooks and guides for the cereal grains wheat, rice, and oats but data for barley are not present (Cooperative Extension Service, 2019). With winter wheat production declining in Arkansas, malt quality barley could serve as an alternative winter small grain for Arkansas producers (USDA-NASS, 2019).

While many different malting grains are available, barley is considered the best for malting, and thus there is potential for barley to be an economically successful crop for Northwest Arkansas. The malting process consists of steeping, germination, and kilning. When looking at kernel characteristics for malting, germination rate is one of the most important as it leads to protein and carbohydrate hydrolysis during malting that also occurs during early growth (Muñoz-Amatriáin et al., 2010; American Malting Barley Association, 2017). When analyzing malt quality, malt factors such as total protein, malt modification, congress wort, and malt enzyme levels are all important (American Malting Barley Association, 2017).

There are currently no recommendations for barley production in Arkansas. The purpose of this study was to determine how harvest date affects the malting quality of barley in Northwest Arkansas. Specifically, we determined the effect of harvest date on barley seed quality characteristics that impact malting and the interaction of harvest date and cultivar. This study provided preliminary data to formulate a recommendation for harvest date in Northwest Arkansas, to suggest variety recommendations, and to aid in future studies on barley in the area.

Materials and Methods

Barley Cultivars and Experimental Design

Five winter malting quality barley cultivars were used for this study including Charles, Endeavor, McGregor, Thoroughbred, and Wintmalt. Of these cultivars, Charles, Endeavor, and Wintmalt are 2-row varieties; McGregor and Thoroughbred are 6-row varieties. The use of both 2- and 6-row varieties was important to evaluate if genetics impacted traits more than the environment. The location in which each cultivar was developed is also important as varieties are bred to perform well in different growing environments and hence affect adaptation to Northwest Arkansas conditions. Charles and Endeavor were developed in Idaho, McGregor was developed in Wisconsin, Thoroughbred in Virginia, and Wintmalt in Europe but adapted to Washington (French, 2012; Obert et al., 2009; Virginia Polytechnic Institute and State University, 2013; Windes and Obert, 2009).

The barley cultivars were drill-seeded in four-row plots at a rate of 250 seed/m² in a randomized complete block design with eight replications on 21 October 2017. Plot dimensions were 1.5 m wide and 1.22 m. Plots were managed using recommended cultural practices for wheat production because there are no current recommendations for barley in Arkansas. Nitrogen fertilizer in the form of urea was applied twice during the study. The first application was 67.25 kg/ha (27 February 2018) and the second was 33.63 kg/ha (21 March 2018).

Trait Measurement

During the season, heading date was recorded on each plot as the day when 50% of the developing barley heads fully emerged from the leaf sheath. A single row from each replication was harvested on four different dates, with the first date beginning at physiological maturity on 1 June 2018 (HD1). Subsequent harvest dates occurred on 8 June (HD2), 15 June (HD3), and 23 June 2018 (HD4). After harvest, samples were oven-dried to a constant moisture of 7.5% and subsequently stored to maintain 7.5% moisture until processing.

In the Fall of 2018 and Spring of 2019, the tests to determine grain yield, test weight, germination capacity, germination energy, and water sensitivity were performed at the University of Arkansas System Division of Agriculture's wheat breeding program lab located on the Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas. Protein analysis was outsourced to the USDA Agricultural Research Service–Cereal Crop Research Unit.

Grain yield was measured by weighing the grain harvested from each plot after cleaning. Measurements were taken by weighing the seeds and envelopes used to store the grain after taking an empty 8-ounce spear envelope.

Test weights were measured by taring a 6000-g scale to the weight of a ¼ cup measuring cup. The ¼ cup measuring cup was then overfilled; a straight edge was run across the top of the measuring cup to ensure the seeds were level with the top edge of the measuring cup. The filled ¼ cup measuring cup was weighed and the weight recorded in grams per ¼ cup. Weights were converted using the following formula $[(\text{weight in g}) \times 1690.7] / 1000 = \text{test weight in kilograms per hectoliter}$ resulting in the reported test weight.

Germination compacity, germination energy, and water sensitivity were measured simultaneously using methods adapted from the Simultaneous Determination of Germination Energy, Water Sensitivity, and Germination Capacity in Barley (Kuester et al., 1997). Four Petri dishes were filled with 2 pieces of filter paper each for every sample to be tested. Next, the dishes were labeled A through D for each sample and 100 seeds for each sample were added to each Petri dish. Four milliliters of distilled water was added to each of the dishes labeled A and B, and 8 mL was added to the dishes labeled C and D. The Petri dishes were then stacked and placed at room temperature in plastic boxes to prevent excessive evaporation of water out of the dishes. Each of the dishes was inspected as close to every 24 hours as possible. When being checked the chitted seeds (seeds with the radical extruding) were considered to be germinated and removed to prevent them from continuing to imbibe water. After 72 hours of germination, the total number of seeds germinated for dishes A and B of each sample were averaged resulting in the germination energy for the sample. The water sensitivity was calculated by averaging the seeds germinated in dishes C and D and subtracting it from the average of A and B, with the resulting formula

$$WS(\%) = \left[\frac{A + B}{2} - \frac{C + D}{2} \right]$$

Following the 72 hours of incubation, all germinated seeds were removed and 2 mL of 0.75% H₂O₂ were added to each of the dishes A and B with seeds remaining. They were then left to incubate for another 48 hours after which the seeds germinated for each dish were counted and the average was taken and reported as germination capacity.

Statistical Analysis

The descriptive statistics mean, median, and standard deviation were calculated in Microsoft Excel. An analysis of variance (ANOVA) was performed in SAS 9.4 with the factors cultivar, harvest date, and the interaction of cultivar and harvest date treated as fixed effects and replication as a random effect (Table 1). Means were separated using Fisher's least significant difference test at $\alpha = 0.05$.

Results and Discussion

Analysis of Variance

The effects of cultivar and harvest date were significant for grain yield, test weight, water sensitivity, germination energy, and germination capacity. There was no significant variation between cultivars for protein content, which ranged from 16.17% to 12.34% on a dry basis. There was an interaction between cultivar and harvest date for water sensitivity, germination energy, and germination capacity.

Barley Grain Yield

Grain yield is important to malting quality barley as greater grain yields result in greater malt being produced from the harvested area. There was a difference in grain yield due to harvest date with HD1 (physiological maturity) at 132 g/plot, greater than all other harvest dates. There was no significant difference observed between harvest dates HD2, HD3, and HD4, which yielded 102, 96, and 106 g, respectively.

Grain yield was also affected by cultivar, with Thoroughbred being the greatest yielding at 185 g and different from all other cultivars. The grain yield of Thoroughbred was nearly double that of Endeavor (96 g), McGregor (95 g), and Wintmalt (95 g). The grain yield of Endeavor, McGregor, and Wintmalt was not different. Charles was lower yielding at 71.97 g (Fig. 1).

Germination Energy

Germination is important to the malting process, so a greater germination energy (GE) is better for achieving a superior malting quality. The expectation is that GE is at 98% or greater for malting quality barley (American Malt-

Table 1. Analysis of variance (*P*-values) for malting traits in five barley cultivars (Charles, Endeavor, McGregor, Thoroughbred, and Wintmalt).

Malting traits	Water sensitivity	Germination energy	Germination capacity	Protein
Cultivar	<0.0001	<0.0001	<0.0001	0.0567
Harvest date (HD)	<0.0001	<0.0001	<0.0001	0.2512
Cultivar x HD	<0.0001	<0.0001	<0.0001	0.8503
Replication	0.0585	0.5801	0.696	0.0188

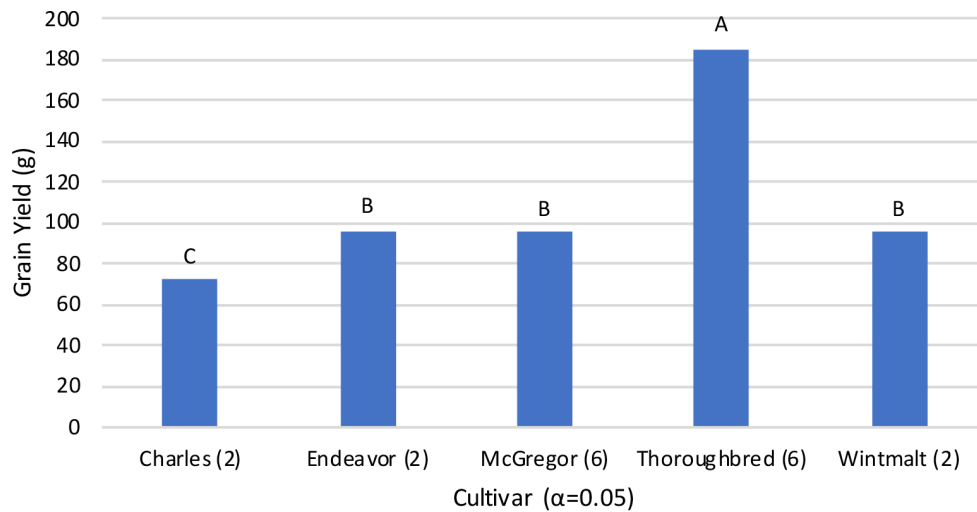


Fig. 1. Effect of cultivar on barley grain yield for cultivars Charles, Endeavor, McGregor, Thoroughbred, and Wintmalt. Means were separated using Fisher's least significant difference test at $\alpha = 0.05$. Numbers in parentheses indicate whether the cultivar is a 2- or 6-row type barley.

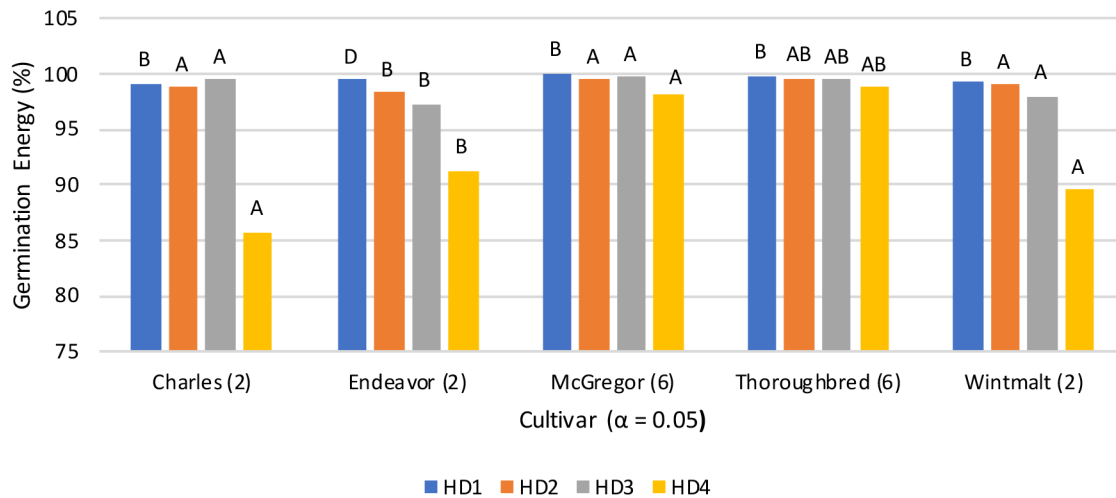


Fig. 2. Effect of cultivar by harvest date interaction on germination energy for five barley cultivars: Charles, Endeavor, McGregor, Thoroughbred, and Wintmalt. Means were separated using Fisher's least significant difference test at $\alpha = 0.05$. Numbers in parentheses indicate whether the cultivar is a 2- or 6-row type barley. HD1 = Physiological Maturity; HD2 = 1 week after Physiological Maturity; HD3 = 2 weeks after Physiological Maturity; HD4 = 3 weeks after Physiological Maturity.

ing Barley Association, Inc., 2019). Germination energy was affected by harvest date, cultivar, and the interaction of cultivar and harvest date. While HD1, HD2, and HD3 were the same, HD4 was 6% lower in germination capacity.

The differences in germination energy between cultivar and harvest date showed that the 6-row cultivars (McGregor and Thoroughbred) stayed relatively consistent across all harvest dates, with McGregor dropping 1.8% and Thoroughbred dropping 1.3% between HD1 and HD4. Germination energy for the 2-row cultivars (Charles, Endeavor, and Wintmalt) was reduced by greater than 5% between HD3 and HD4. Before that, the germination energy of Endeavor and Wintmalt dropped 2.3% and 1.3%, respectively, between HD1 and HD3. However, Charles held steady at approximately 99% through the first three harvest dates and dropping on the HD4 (Fig. 2).

Germination Capacity

The measure of the number of seeds that germinated after exposure to 0.75% H_2O_2 quantified germination capacity. This capacity reflects the ability to germinate in non-optimal conditions. Therefore, greater germination capacity is desired for malting quality barley. Overall, the trend for germination capacity was similar to germination energy across harvest dates, cultivars, and cultivar by harvest date (data not shown).

Water Sensitivity

Water sensitivity (germination in 4 mL of water compared to germination in 8 mL water) differed between harvest date, cultivar, and cultivar by harvest date. Harvest date 1 showed the least sensitivity to water, at less than 1%, and HD4 is the most sensitive at 9%. Harvest date 2 and HD3 were not different (Fig. 3). The 6-row varieties, McGregor and Thoroughbred, were lower in water sensitivity than the 2-row varieties making them more desirable for malting (Fig. 3).

Except for Endeavor, all cultivars were at or below 1% water sensitivity for HD1. For HD2, Charles and McGregor were both near 2% with Charles being 2.4% and McGregor being 1.9%. Water sensitivity for Thoroughbred, Endeavor, and Wintmalt also increased for HD2 with Thoroughbred increasing to almost 4%, Endeavor to 4.5%, and Wintmalt to 8%. However, HD3 was different with Thoroughbred staying near 4%, and the 4 other varieties increasing. Charles increased to 5% from 2%, Endeavor to 6.5% from 5%, McGregor to 3% from 2%, and Wintmalt to over 10% from 8%. Harvest date 4 had the greatest sensitivity for 4 of the 5 cultivars. The sensitivity for Charles and Endeavor increased by more than 8%. McGregor increased to 4% and Thoroughbred recorded its greatest water sensitivity at 4%. Wintmalt was the only cultivar for which the sensitivity decreased for the 4th harvest date dropping from 10% to 7.5% (Fig. 3).

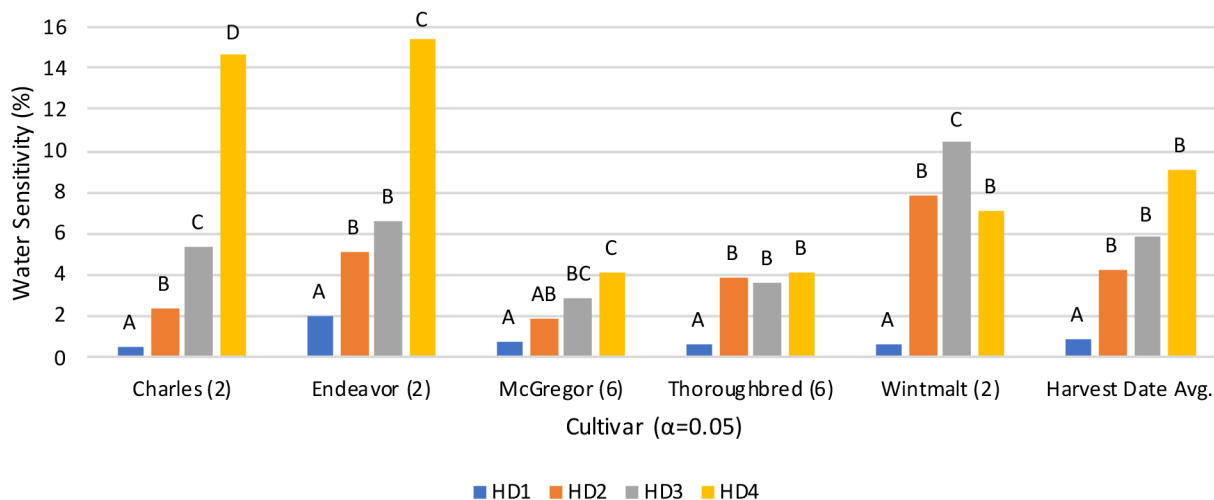


Fig. 3. Effect of cultivar by harvest date interaction on water sensitivity for five barley cultivars with harvest date averages: Charles, Endeavor, McGregor, Thoroughbred, and Wintmalt. Means were separated using Fisher's least significant difference test at $\alpha = 0.05$. Numbers in parentheses indicate whether the cultivar is a 2- or 6-row type barley. HD1 = Physiological Maturity; HD2 = 1 week after Physiological Maturity; HD3 = 2 weeks after Physiological Maturity; HD4 = 3 weeks after Physiological Maturity.

Conclusions

The optimal harvest date was at physiological maturity for malting quality barley. Many of the malting traits (such as germination energy) declined as harvest was delayed. It is possible to minimize the losses to the malting traits if harvest is completed within 14 (HD1 to HD3) days of maturity but by 21 days after maturity, it is unlikely to meet the criteria for malting quality barley. Of the cultivars tested, the 6-row cultivar, Thoroughbred, performed better than other cultivars. It has the greatest grain yield and thousand kernel weight, also it performed the most consistently across the malting traits maintaining malting quality germination energy and capacity into HD4. Thoroughbred became more sensitive to water by HD4, so harvesting early is still recommended. Of the 2-row cultivars, Wintmalt performed consistently but was more prone to reduced performance as harvest was delayed. McGregor had slightly lower yield but better performance in the malting traits than Wintmalt. For malting quality barley, the recommended cultivars are Thoroughbred and McGregor followed by Wintmalt for the traits evaluated in this study. While Thoroughbred came the closest to meeting the criteria for malting quality, all the cultivars tested failed to meet the requirement for malting quality with protein: Charles and Wintmalt having protein contents of 15%, and McGregor, Thoroughbred, and Endeavor having 14%, all higher than the 13.5% maximum. Further studies to evaluate additional cultivars with genetically lower protein in combination with cultural practices that limit grain protein content, while not sacrificing grain yield, will be important for the production of profitable malting quality barley in the state.

Acknowledgements

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Acknowledgements

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Written comments of reviewers will be provided to the author usually via track changes through Word. Student authors are expected to make revisions as part of the publication process. Students will be required to submit a separate file stating how each comment was addressed in the revision. If the student author disagrees with a suggestion, the rationale for not making a suggested change should be provided.

View an example of a response to reviewer document at:

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When a paper is accepted “with revisions,” a revised manuscript will need to be submitted through ScholarWorks@UARK and the managing editor will approve a final draft for publication.

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About the Dale Bumpers College of Agricultural, Food and Life Sciences

Bumpers College provides life-changing opportunities to position and prepare graduates who will be leaders in the businesses associated with foods, family, the environment, agriculture, sustainability and human quality of life; and who will be first-choice candidates of employers looking for leaders, innovators, policy-makers and entrepreneurs. The college is named for Dale Bumpers, former Arkansas governor and longtime U.S. senator who made the state prominent in national and international agriculture.

About the University of Arkansas System Division of Agriculture

The University of Arkansas System Division of Agriculture's mission is to strengthen agriculture, communities, and families by connecting trusted research to the adoption of best practices. Through the Agricultural Experiment Station and the Cooperative Extension Service, the Division of Agriculture conducts research and extension work within the nation's historic land grant education system. The Division of Agriculture is one of 20 entities within the University of Arkansas System. It has offices in all 75 counties in Arkansas and faculty on five system campuses.

